# *Journal of*
# DAIRY SCIENCE

Vol. 43 May, 1960 Number 5


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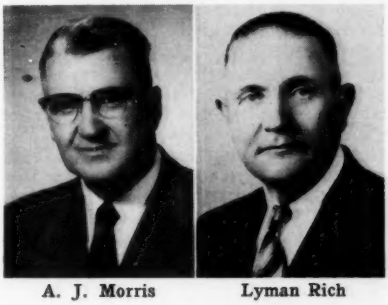
# PEOPLE AND EVENTS

### Utah Plans Attractive Program for A.D.S.A. Visitors

Dairy scientists of the nation who plan to attend the annual meetings of the American Dairy Science Association at Utah State University, June 19-22, will receive a warm welcome to one of the few typical college communities in the country.

A. J. MORRIS, head of the Utah State University Dairy Science Department, is chairman of conference arrangements and LYMAN RICH is chairman of the Steering Committee. MRS. LYMAN RICH, MRS. DAVID BURGOYNE, and MRS. A. J. MORRIS are in charge of program arrangements for the women and children.



A. J. Morris Lyman Rich

The arrangements committee has planned an attractive program of activities for men, women, and children. Features include: an address by the HONORABLE EZRA TAFT BENSON, Secretary of Agriculture, a concert by the famous Salt Lake Tabernacle Choir, and a chicken barbeque for all; scenic and historic tours of the Valley and Canyon, luncheon and president's tea for the ladies; swimming, hiking, and other supervised recreation for young people; nursery school for the little ones; and over 200 papers on important dairy research from 42 states, together with important symposia.

Events of the University's summer school are being scheduled now to give visitors to the A.D.S.A. meetings a free rein on facilities of the University, and business and civic leaders of the community are wholeheartedly cooperating to assure a pleasant stay.

Logan, site of one of seven Mormon Temples of the Church of Jesus Christ of Latter-Day-Saints, is a city of fine schools and fine churches, with Latter-Day-Saints, Protestant, and Catholic groups well represented.

Utah State University's campus is situated on the eastern slopes of beautiful Cache Valley, so named because it was here that early-day trappers and mountain men cached their furs.

To assist in the full enjoyment of the University and the city, and its surrounding recreational and scenic areas, the Cache Chamber of Commerce is creating a special tourist brochure, complete with maps and pictures and featuring local and other intermountain tours to Bear Lake, the Grand Tetons, Jackson Lake, Yellowstone, Zion, Bryce and Grand Canyon National Parks, and the great Salt Lake. These are many of the places that delegates will want to visit en route to or from the dairy meeting.

Cache Valley's annual Dairy Festival is planned for Friday and Saturday preceding the meetings and all are invited to participate in the program. In addition to a parade, the Festival will feature visits to and exhibits by the world's largest Swiss Cheese factory, dairies, and other processing plants affiliated with the county's major industry.

Come to Logan and Utah State University. Everything is being done to assure the full enjoyment of the meetings, the extracurricular activities, and the many recreational and scenic spots where you can spend from one hour to several days, enjoyable and profitably.

### Dairy Industries Supply Association Honor Plaque Goes To Roberts Everett for Four Decades of Distinguished Service

ROBERTS EVERETT, Executive Vice-President of Dairy Industries Supply Association, was the recipient of the 12th DISA Honor Plaque at DISA's 41st Annual Meeting, March 31, 1960, at the Edgewater Beach Hotel in Chicago.

The veteran supplier-equipper executive, who came to work for DISA in 1919, will step into semi-retirement in July. The DISA Honor Plaque is awarded by the DISA Board to those persons who have made exceptional contributions to the trade association. In making the presentation, D. G. COLONY, Manton-Gaulin Manufacturing Company, Inc., DISA's President, said:

"DISA was chartered in 1912, and struggled along as an informal club until 1919, when a young airforce veteran and newspaperman named Roberts Everett came along to breathe life into the loose confederation of firms selling to the ice cream industries. Through his executive leadership and devotion, DISA grew into the indispensable arm to all of us that it is.

"Not a single activity undertaken by the association has ever failed to have his closest attention. His mind has grasped a thousand divergent strains of thought in one instant, and translated them into understanding for all of us.

"This is the last annual meeting Bob will attend in his role as Executive Vice-President; he will step into semi-retirement this coming July. I say semi-retirement, because he will continue to serve DISA as a consultant for a

contracted period of years, and, I hope, many years more.

"More than any other person's, DISA is Roberts Everett's monument."

**The Honor Plaque—highest award from the Dairy Industries Supply Association for outstanding service—is presented to Roberts Everett (right), Executive Vice-President of DISA since 1919. Chairman of DISA's Executive Committee, D. G. Colony, makes the presentation at the 41st Annual Meeting, March 31, at the Edgewater Beach Hotel in Chicago.**

Mr. Everett was also presented a parchment citation by the Board of Directors, containing a resolution saluting his record of service. This presentation was made by S. E. CROFTS, Batavia Body Company, Incorporated, DISA's Chairman of the Board.

The Honor Plaque of the association has been presented to only 11 individuals, three of whom are deceased. They are: JOHN H. MULHOLLAND, DISA's long-time treasurer and honorary president for life (deceased); K. L. WALLACE, DISA president during the Korean War period (deceased); EARL WEED, a former director and leading committee worker (deceased); L. N. LUCAS, past-president; GORDON LAMONT, past-president; HARRY MILLER, past-president; RAY OLSON, past-president; ROBERT ROSENBAUM, past-president; G. E. WALLIS, past-president; DICK BRITTON, vice-president and committee worker; R. L. YOUNG, long-time treasurer.

### Dairy Products Specialist Named At Minnesota

V. S. PACKARD, a research worker in dairy industries at the University of Minnesota since 1954, has been named dairy products specialist for the University's Agricultural Extension Service. He will work closely with the dairy industry, county agents, and dairy farmers on quality improvement of dairy products. Packard earned the B. S. degree from the University of Maine in 1954, the M. S. at the University of Minnesota in 1956, and is completing work toward a Ph.D. degree. He has conducted research on ice cream packaging for self-service merchandising and quality control in other dairy products. He has taught courses in testing and technical control of dairy products at the University. He replaces J. H. GHOLSON, who left the University in 1959.

### ONTARIO AGRICULTURAL COLLEGE NEWS

The first Canadian Soft Ice Cream Operators Conference was held in the Dairy Department, Ontario Agricultural College, Guelph, March 14-15. The conference was organized by A. M. PEARSON, and 107 people attended it. Speakers included: J. KEAY of the Canada Department of Agriculture Marketing Service; P. G. KEENEY of the Pennsylvania State University Dairy Dept.; J. H. KAPPUS, The Taylor Freezmaster Company; A. G. LEGGATT of the O.A.C. Dairy Dept., and G. W. FUNSTON, Chairman of the Canadian Dairy Industry Suppliers Association Committee on Flavors and Toppings. The success of the conference should make it an annual event.

---

The 1950 3-mo. Dairy Short Course has been completed. Twenty-three students from four Canadian provinces and four foreign countries attended the course, which covered fundamental and practical procedures in manufacturing and testing of dairy products, dairy chemistry, dairy plant management, and dairy bacteriology.

---

The Ontario Milk Distributors have awarded the O. A. C. Dairy Dept. a $1,000 grant-in-aid for a 6-mo. study of the freezing point of milk in Ontario.

The Ontario Association of Ice Cream Manufacturers has presented a $250 gift of books for the Dairy Department Library at O. A. C.

### West Virginia University Holds Dairy Cattle Judging School

The 1960 Dairy Cattle Judging School, sponsored by the Dairy Department of West Virginia University, and held April 1-2, was declared the best yet by the 50 men who participated in it. The School is held every other year, to give practice in the art of judging, for certain county agents, Vo-Ag teachers, dairy farmers, and herdsmen who judge at local and county fairs throughout the State.

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A new feature this year was an evening session on The Evaluation of Pedigrees, conducted by I. D. PORTERFIELD and R. G. MITCHELL. Interest at this meeting was high, and it brought forth many questions and much discussion.

Judge for the two-day affair was EDSEL GAINER, Extension Dairyman at the University of Maryland. Mr. Gainer is a native West Virginian and a dairy graduate of West Virginia University. His placings and remarks following each class were well received by the group.

## IOWA NEWS

### Arnold Assumes Foreign Post

F. J. ARNOLD, Ames, Iowa, an extension dairy specialist for nearly 33 yr., has resigned this position to join the staff of the International Cooperation Administration (ICA). He left Ames on March 20 for 3 wk. of indoctrination in Washington, D. C., before moving with Mrs. Arnold to Bangkok, Thailand, where he will serve as assistant chief of the agricultural division of the U. S. mission to that country.

A native of Hornick, Iowa, Professor Arnold graduated from Iowa State in 1926, and received a master's degree from the same institution in 1940. He joined the Iowa State extension staff in 1927, where he has served continuously except for 4 yr. as extension dairyman in Maryland in 1943-47.

In 1955, he received the DeLaval Extension Dairyman Award at the American Dairy Science Association meeting. He has been active in A. D. S. A. work, and for many years has worked closely with the National Dairy Cattle Congress at Waterloo.

## NEWS FROM WISCONSIN

B. R. BAUMGARDT is a new addition to the Dairy Husbandry staff at the University of Wisconsin, taking the place of the late N. N. ALLEN. Assistant Professor Baumgardt comes to Wisconsin from Rutgers University. At Wisconsin his duties are teaching and research in the areas of dairy husbandry and ruminant nutrition. Research emphasis is on factors involved in efficient use of high-quality forage by dairy cattle.

Dr. Baumgardt received his B. S. from Purdue, and the M. S. degree in 1955 from the same institution, working under a Ralston-Purina fellowship. He went to Rutgers University in June, 1956, as instructor and research associate. There he taught several

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general dairy husbandry courses, coached the dairy cattle judging team, and worked with dairy cattle nutrition research. He received his Ph.D. in Agricultural Biochemistry in June of 1959. His research was on comparison of in vivo and in vitro rumen digestion procedures, as applied to an estimation of forage quality.

## CLEMSON COLLEGE NEWS

### Quality Control Conference

A Milk Quality Control Conference was held recently on the Clemson College campus, sponsored by the South Carolina Dairy Association and the Clemson College Dairy Department. A total of 65 were in attendance. Dairy plants in neighboring states of North Carolina and Georgia were represented.

The following topics were discussed by staff members: What Is Quality Control?, Quality Control at the Producer Level, Quality Control in the Processing Plant, Evaluation of a Quality Control Program, Observations on Quality Control in South Carolina.

Quality control from the processing plant to the consumer was discussed by a panel composed of five members from industry. Demonstrations on antibiotic tests and the TESA butterfat test were presented.

---

A Conference on Antibiotic and Pesticide Residues in Milk was held April 12-13 in the Food Industry Building on the Clemson College Campus. This Conference was sponsored jointly by the University of Georgia and Clemson College Dairy Departments, with the cooperation of the South Carolina Dairy Association and the Georgia Milk Commission.

The welcome address was given by DEAN M. D. FARRAR of Clemson College, and the following program was given: History and Review of the Problem of Antibiotic and Pesticide Residues in Milk. J. J. SHEURING, Professor of Dairy Husbandry, University of Georgia, Athens; The Health Hazard and Health Department Attitudes and Policy Concerning Antibiotic and Pesticide Residues in Milk. W. R. MCLEAN, Regional Milk and Food Consultant, Public Health Service, Department of Health, Education and Welfare, Atlanta, Georgia; What the Dairy Industry Can and Must Do to Prevent Antibiotic Residues in Milk. H. K. WELCH, JR., Project Leader, Dairy Extension Work, University of Georgia, Athens; What the Dairy Industry Can and Must Do to Prevent Pesticide Residues in Milk. T. R. ADKINS, Assistant Professor of Entomology, Clemson College; Antibiotics and Pesticides in Milk. J. J. MILUNAS, Assistant Director, Atlanta District, Food and Drug Administration; Methods That Can Be Effective in Controlling Pesticide Residues in Milk, a series of short résumés by representatives

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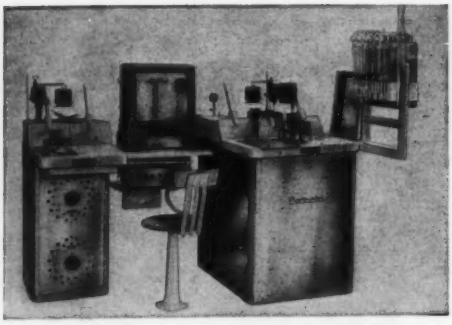

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from industry and State Boards of Health, and Methods for the Detection of Residual Antibiotics and Pesticides in Milk. Evaluation of Usefulness to the Dairy Industry. R. W. HENNINGSON, Assistant Professor of Dairying, Clemson College.

---

The Eighth Annual Meeting of the Southeastern Dairy Marketing Clinic was held at the Clemson House, Clemson, S. C., March 28-29.

High lights of the program were: Legislation — Taxation — Research: Taxation—DONALD E. HIRSCH, American Farm Bureau Federation, Chicago, Illinois; Legislation—MACK GEBERT, National Milk Producers Federation, Washington, D. C.; Research—D. H. JACOBSEN, Director Research and Education, American Dairy Association, Chicago, Illinois.

Under the subject of State Milk Control Programs, a panel discussion was carried on by the following: G. E. HAWKINS, SR., Secretary, S. C. Council of Milk Producer Assns., Greenwood, S. C.; C. A. SHAW, Director, S. C. Dairy Commission, Columbia, S. C.; JACK WHITTAKER, Secretary, N. C. Dairy Commission, Raleigh, N. C.; G. C. BURROUGHS, Chairman, Virginia State Milk Commission, Roanoke, Virginia.

Moderator of the program on Surplus—Whose Problem Is It? How Best to Meet the Problem, was FRANK MCDOWELL, Acting Mgr., Carolina Milk Producers Assoc., Greensboro, N. C. Panel members were: GLENN SUMMERS, Mgr., Nashville Milk Producers Assoc., Nashville, Tenn.; B. F. MORGAN, JR., Mgr., Tri-State Milk Producers Assoc., Bristol, Virginia; J. K. EARLE, JR., Dairyman, Greenville, S. C.; E. H. AGNEW, Pres., South Carolina Farm Bureau, Columbia, S. C.

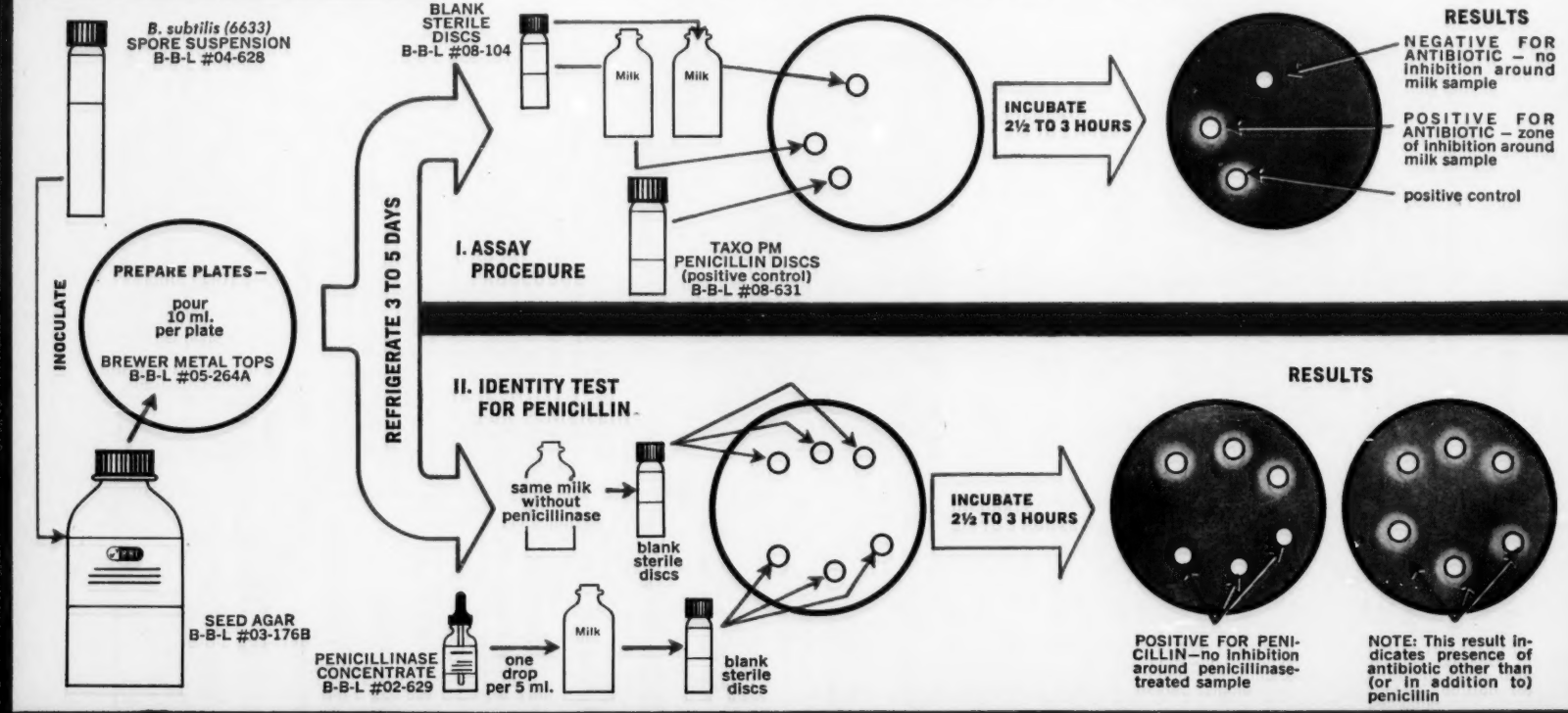
Moderator of the program on Stronger, More Effective Cooperatives, was F. L. HAMMACK, Executive Secretary, Palmetto Dairyman's Federation, Inc., Greenville, S. C. Panel members were GLENN LAKE, Vice-Pres., National Milk Producers Federation; and G. H. AULL, Head, Agricultural Economics, Clemson College, Clemson, S. C.

## OHIO STATE UNIVERSITY NEWS

The Ninth Annual Milk Sanitation and Public Health Short Course, sponsored cooperatively by the Department of Dairy Technology, The Ohio State University, and the Ohio State Departments of Health and Agriculture, was held March 14-18 on the O. S. U. Campus. A capacity number of students participated in the course, which is designed for milk sanitarians and dairy plant employees. The subjects dealt with the fundamentals of dairy farm and dairy plant inspections, sanitation practices, dairy farm and dairy plant equipment design and operation, public

B-B-L TECHNICHART

# DETECTION OF PENICILLIN IN MILK




**The presence of antibiotics in milk** following mastitis therapy in cows has created serious public health problems and caused technical difficulties within the dairy industry. A rapid, practical laboratory procedure to assist regulatory agencies and the dairy industry in solving these problems was described by Arret and Kirshbaum.* This procedure employs rapid growth of a sensitive strain of *B. subtilis* for assaying the presence of antibiotics in milk and for determining its identity with penicillin. Inhibition of growth by the presence of as little as 0.05 unit of penicillin per ml. of milk sample is detectable within 2½ hours.

In answer to many requests for information about the availability of B-B-L products for this simplified procedure, the B-B-L Development Laboratory has prepared this TECHNICHART. It graphically illustrates the basic procedure, showing the materials necessary—**all of which are available from B-B-L.** A complete brochure with detailed technique and product listing is available upon request.

*Arret, B., and Kirshbaum, A.: J. Milk and Food Technol. 22:329, 1959.


**BALTIMORE BIOLOGICAL LABORATORY, INC.**
**BALTIMORE 18, MARYLAND**
A Division of Becton, Dickinson and Company


B-B-L


B-B-L AND TAXO ARE TRADEMARKS. 81560

health problems, legal standards and milk ordinances, and interpretation of regulations.

The keynote address, Regulations—Aids or Hindrances to the Dairy Industry was delivered by H. E. ROBINSON, Chief of the Milk Program, USPHS, Washington, D. C. Other visiting speakers included R. B. DOUGLAS, Beatrice Foods Co., New Bremen, Ohio; D. A. SEIBERLING, Klenzade Corporation, Beloit, Mich.; C. CROSBY, DeLaval Separator Co., Van Wert, Ohio; RALPH PALMER and J. H. ERB, Borden's, Columbus; L. C. BURKEY, Creamery Package Corp., Toledo, Ohio; J. R. KELSEY, Cherry-Burrell, Columbus; KEITH BERG, Central Ohio Milk Producers Assoc., Columbus; F. C. FUZZARD, Taylor Instrument Companies, Cleveland, Ohio; K. T. YODER, Diamond Milk Co., Columbus, J. O. ROBERTS, D & R Laboratories, Columbus; L. C. PECKHAM, USPHS, Chicago, Illinois; and C. K. ORTMAN, M & R Dieteties, Columbus.

Coordinators of the program were H. E. KENNEDY, Department of Dairy Technology, The Ohio State University; RAY WATTS, State Milk Sanitarian, Ohio State Department of Agriculture, Columbus; and O. L. HUNNICUTT, Division of Dairy and Foods, Ohio State Department of Agriculture, Columbus.

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W. J. HARPER, Associate Professor, Department of Dairy Technology, The Ohio State University, will be on combined sabbatical and assigned research leaves, effective July 1 through December 31, 1960, from The Ohio State University and the Ohio Agricultural Experiment Station. Dr. Harper will use the major portion of his leave to conduct research in the Dairy Department of the Alnarp Institute, Sweden. In addition, he will visit the Hannah Dairy Institute, Scotland, and the Dairy Research Institute at Kiel, Germany; Reading, England; and Wagenaar, Holland. Dr. Harper will be accompanied by his family.

**Back of Beacon Dairy Feeds . . .**

- Qualified Research Personnel
- Research and Demonstration Herd (45 cows—15,634 M, 3.6%, 568 F, H.I.R.)
- Special Research Herd of 12 sets of identical twin females
- 40 Years Experience in Practical Dairy Feed Formulation

**BEACON FEEDS**

THE BEACON MILLING COMPANY
A division of Spencer Kellogg and Sons, Inc.
CAYUGA, N. Y.

## NEWS FROM WYOMING

A very successful Dairy Manufacturing Short Course was conducted at the University of Wyoming at Laramie, February 12-13. Attendees numbered 58, and the speakers covered their subjects with competence and interest—two factors making for the success of the Course. Products contests for milk, and vanilla and chocolate ice cream were also conducted. (See photo.) Prizes for these contests were supplied by various Dairy Industry suppliers. Of the 12 prizes awarded in the products contests, six were won by Wyoming dairies.

During the meeting, those in attendance formed an organization to be known as the ROWDISCA (Royal Order of Wyoming Dairy Industry Short Course Attenders). Requirements for membership are attendance at a short course. The group is more of a fun club than a serious organization, but it should have a wholesome effect on the morale of the industry in Wyoming.

## CHERRY-BURRELL CORP. NOTES

Appointment of E. L. SAVONEN as Manager of the St. Paul Sales Branch for Cherry-Burrell Corporation, effective April 1, has been announced by J. R. FRYE, Assistant General Sales Manager for the Company.

Gene Savonen joined Cherry-Burrell in 1949 as a branch engineer trainee. A year later, he was assigned to Cherry-Burrell's St. Paul

# HOW KRAFT SERVES THE DAIRY INDUSTRY WITH STABILIZERS AND EMULSIFIERS

Kraft offers the dairy industry a wide variety of these ingredients for manufacturing ice cream, sherbets and frozen novelties. Each Kraft stabilizer and emulsifier is produced to meet Kraft's high standards and is designed to fill a specific dairy need. Accordingly, each helps insure that the dairy's end product will be of uniform goodness.

**STABILIZERS FOR ICE CREAM**

KRAGELEEN ICE CREAM STABILIZER. Used in any ice cream mix for vat pasteurization or HTST where the mix is preheated. Made from Kraft's own specially processed Irish moss and designed to properly stabilize the mix and give it good body and a creamy melt-down.

KRAGELEEN FL. A special formula, developed specifically for HTST, to produce a top-quality ice cream.

**STABILIZER FOR SHERBETS**

SHER-STAY SHERBET STABILIZER. A new Kraft development for stabilizing sherbet, Sher-Stay reduces spurting and improves texture and body.

**STABILIZER FOR FROZEN NOVELTIES**

KRABYN STABILIZER. In new instant form . . . an excellent stabilizer for frozen novelties, designed to produce a smooth texture, proper viscosity and prevent the growth of ice crystals.

**EMULSIFIER FOR ICE CREAM**

KRAFT'S ICE CREAM EMULSIFIER. Kraft Emulsifier for ice cream and frozen confections. The 100% emulsifier that gives whipability to ice cream without interfering with efficient freezing. It improves texture and makes a dry ice cream.

**EMULSIFIER-STABILIZERS FOR ICE CREAM**

KRAFT EMULSIFIER-STABILIZER. This combination product functions as both a stabilizer and an emulsifier and offers the advantage of handling only one ingredient. It allows freezing the mix immediately after processing and gives better control of over-run.

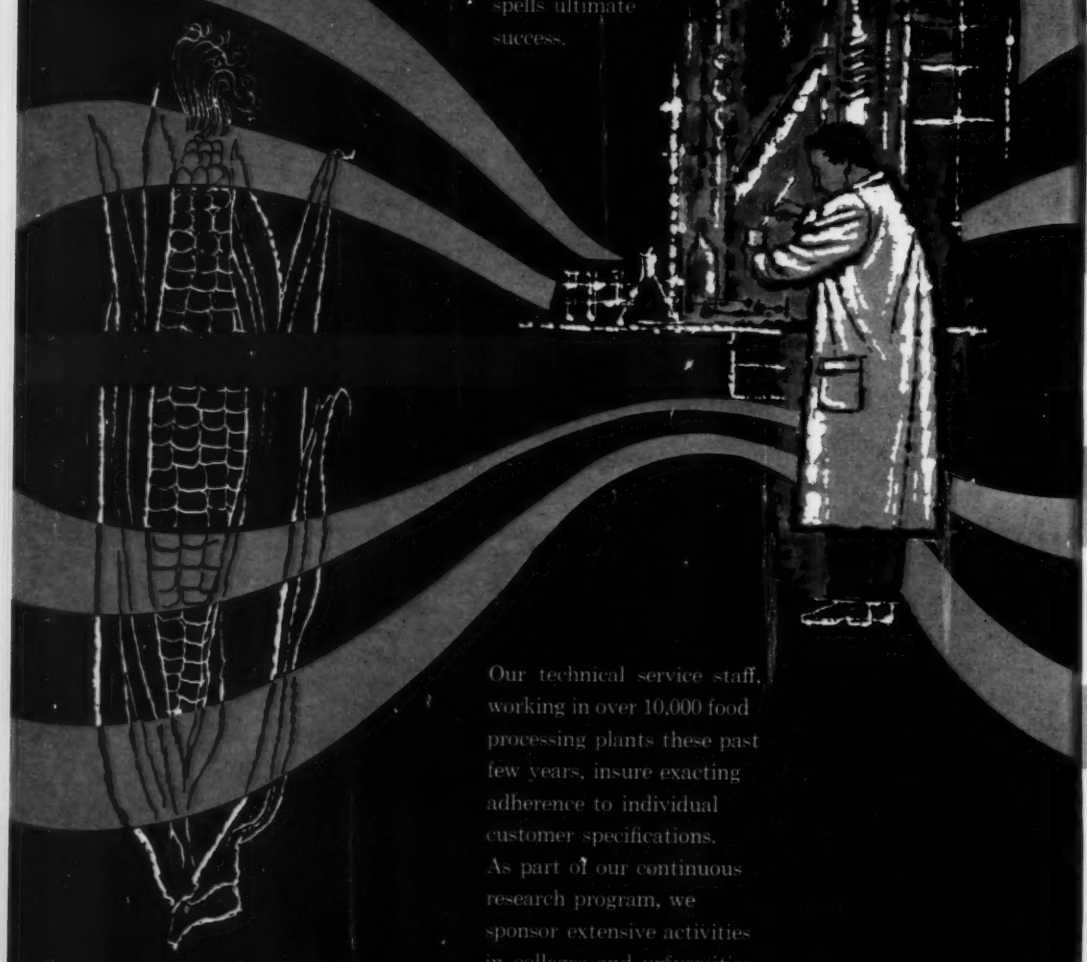
KRAFT 17 ES. Combining the advantages of both a stabilizer and an emulsifier, this ingredient produces a dry ice cream, controls over-run and is uniform and economical.

KRAFT

To obtain free samples for classroom work, ask your Kraft Man or write
KRAFT FOODS DAIRY SERVICE DIVISION
CHICAGO • NEW YORK • GARLAND, TEXAS • SAN FRANCISCO

# INTEGRATION—
# THE INDEFINABLE
# ELEMENT

Success in any corn refining operation depends on many equally vital factors: progressive corn agronomy at the farmer level; sound purchasing policies; specially engineered equipment and highly developed personnel at every level of operation. But, it is the balanced integration of all these factors that spells ultimate success.



Our technical service staff, working in over 10,000 food processing plants these past few years, insure exacting adherence to individual customer specifications. As part of our continuous research program, we sponsor extensive activities in colleges and universities.

**AMERICAN MAIZE-PRODUCTS COMPANY**

# AMAIZO®

**FRODEX® or CORN SYRUP**

## GIVES ICE CREAM BUILT-IN PROTECTION AGAINST HEAT SHOCK

From ice cream plant right to the dinner table, AMAIZO FRODEX or CORN SYRUP guards ice cream against frequent handling and too-high storage temperatures, preserving its frosty, "just-made" goodness. Pioneered by American Maize, this outstanding stabilizer for all types of frozen desserts (ice creams, sherbets, water ices and frozen novelties) also gives smoother body and sparks flavor. It adds to the nutritive food solids content. Impressive savings in production are possible, too. Manufacturers who prefer liquid handling use AMAIZO CORN SYRUP; those who prefer dry handling use AMAIZO FRODEX.

*For full information write our Technical Sales Service Staff in New York or any of our sales offices, without obligation, of course.*

ATLANTA • BOSTON • BUFFALO
CHICAGO • DENVER • GRAND RAPIDS • HOUSTON • LOS ANGELES
PHILADELPHIA • PITTSBURGH • ST. LOUIS
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**AMERICAN MAIZE-PRODUCTS COMPANY**
250 PARK AVENUE • NEW YORK 17, N.Y.


Branch, one of the Company's 16 sales offices. Since that time, Savonen has handled both engineering and field sales assignments. He earned his degree in mechanical engineering at the University of Colorado.

Savonen and his wife, Naomi, and their two daughters, Carol, 6, and Lynn, 3, live at 3965 North Fairview Avenue, St. Paul.

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The USSR has contracted to purchase its first automated continuous butter processing system from the Cherry-Burrell Corporation in Cedar Rapids, Iowa.

This $120,000 system is scheduled for shipment in June. It will have a capacity of 2,000 lb. of butter per hour.

In 1938, Cherry-Burrell first sold Vogt ice cream freezers to Russia.

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L. P. MARTENS, Cherry-Burrell Corporation, died in February, 1960, following a brief illness.

Mr. Martens started with Cherry-Burrell in February, 1946, following service in the army as a Major in World War II. He had been in the Sales Department as a machinery specialist, a product sales supervisor, and as a product sales manager in Resale Products Department. Prior to coming with Cherry-Burrell, he was educated in Chicago and held a degree in civil engineering from Armour Institute of Technology (now Illinois Institute of Technology). His survivors are his widow, Alice; a daughter, Mrs. Elizabeth Maddox; and a son, Roy, Jr.

### CREAMERY PACKAGE PROMOTES D. C. ROAHEN TO DIRECTOR OF RESEARCH

D. C. ROAHEN has been promoted to Director of Research of The Creamery Package Mfg. Company, Chicago, according to a recent announcement by the firm's president, E. B. LEHRACK. Mr. Roahen will continue to headquarter at Fort Atkinson, Wisconsin.

Mr. Roahen graduated from Ohio State University with a B. S. degree in Dairy Technology, and received an M. S. degree in Dairy Industry from the University of Wisconsin.

After practical experience in general dairy processing operations with a nationally known company, Roahen joined Creamery Package in 1939 and completed the Company's training program.

He then worked as a sales engineer in the firm's Chicago branch; later handled priorities and government regulations; was transferred to Production Control at CP's Fort Atkinson, Wisconsin, plant; and in 1949 was assigned to the Research Department. In 1957, he was made Senior Research Engineer, from which position he has just been promoted to Director of Research, succeeding A. H. WAKEMAN,

IN THE
DAIRY FIELD
**IODINE SANITIZERS**
OFFER ALL THESE
ADVANTAGES

**A LONG RECORD OF DEPENDABILITY.** Iodine is recognized as a most efficient antiseptic and germicide. It is known to be effective against a wide range of organisms. New technology has now resulted in more efficient iodine formulations developed especially for sanitization.

**EASY TO USE.** Iodine sanitizers are formulated especially for treatment of dairy utensils and equipment. Leading manufacturers offer iodine sanitizers and detergent-sanitizers as liquids, powders or tablets.

**EFFECTIVE.** Iodine sanitizers are effective in low concentrations . . . economical, too. They can help you supply better milk.

**EASY TO TEST.** The well-known iodine color is an indication of solution strength. When the color of an iodine sanitizing solution begins to disappear, that is a signal to replenish or replace the solution. Test kits are available.

**Write us for further information and names of manufacturers offering iodine sanitizers in your area. No obligation, of course.**

CHILEAN IODINE
EDUCATIONAL BUREAU,
INC.

Room 2156
120 Broadway, New York 5, N. Y.

who has been elected Vice-President in charge of Engineering and Research.

Mr. Roahen resides in Fort Atkinson with his wife and three sons. He is active in community affairs and is currently treasurer of the Fort Atkinson School Board.

### BORDEN OPENS NEW PITTSBURGH PLANT

An ultramodern, million-dollar ice cream plant designed to provide smooth, continuous flow of supplies and to avoid all cross-traffic during the production cycle has been opened in Pittsburgh, Pennsylvania, by the Borden Company.

The one-story building, with 43,000 square feet of floor space, is located on a 13½-acre plot on Mansfield Avenue overlooking the Penn-Lincoln Highway. It replaces a Borden plant used for many years at 4410 Liberty Street.

High light of the new plant is its design, which allows for the most efficient use of automatic mixing equipment, a hardening tunnel that handles 1,200 gal. of ice cream per hour, a novelty machine that produces 1,183 units at a time, and the palletizing of supplies and finished products.

### DAWE'S LABORATORIES APPOINTS R. H. JOHNSON

Appointment of R. H. JOHNSON to its technical service department staff as nutritionist has been announced by Dawe's Laboratories, Inc., Chicago.

R. H. Johnson

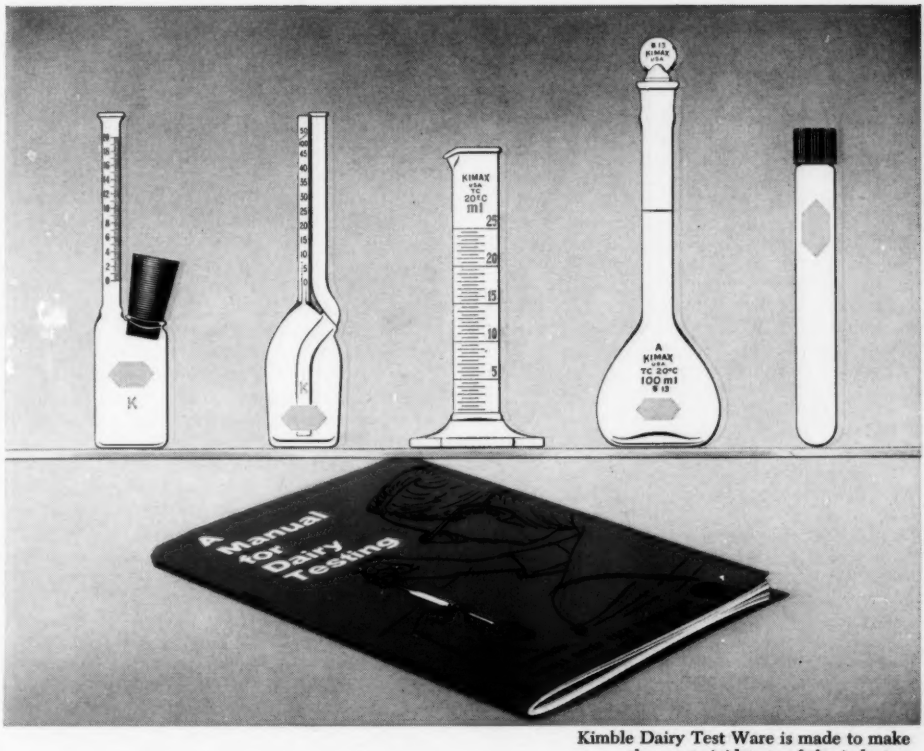
In his new position, Dr. Johnson will work with other Dawe's nutritionists in providing nutritional counseling and other technical services to feed manufacturers.

Before joining Dawe's, Dr. Johnson served as a specialist in beef cattle nutrition in Argentina under the auspices of the United States government's ICA program.

A native of Los Angeles, California, Dr. Johnson received his B.S. degree in dairy husbandry at California State Polytechnic College and his doctorate in animal nutrition at Iowa State University, Ames.

### Pictures of Registered Dairy Cattle To Be Preserved

Strohmeyer & Carpenter, Inc., White Plains, N.Y., will transfer the most valuable picture records of registered dairy cattle in the United States to the Van Dervort Studio, Cobleskill, N.Y., on April 15. The file contains more than




Kimble Dairy Test Ware is made to make the most rigid tests of the industry.

## It's Kimble Dairy Test Ware

### ...the standard for long, dependable service

Outstanding accuracy and quality have established Kimble Dairy Test Ware as a recognized standard of the dairy industry. Careful tempering to equalize strains creates glassware of maximum strength and longest life expectancy.

Kimble Dairy Test Ware meets all federal and state requirements, passes the most rigid tests for accuracy and serviceability.

Kimble Dairy Test Ware is distributed by leading dairy supply houses throughout the United States and Canada.

**FREE!**

**New 5th Edition of "A Manual for Dairy Testing." Send in the coupon for your copy now!**

Kimble Glass Company, Subsidiary of Owens-Illinois, Toledo 1, Ohio
Send new 5th Edition of "A Manual for Dairy Testing."

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GENERAL OFFICES • TOLEDO 1, OHIO

130,000 livestock pictures over the past 44 yr.

H. A. STROHMEYER, JR., and C. T. CARPENTER, JR., partners since 1928, as individuals will continue their activities in photography of dairy animals. However, the Van Dervort Studio will produce all duplicate prints from Strohmeyer and Carpenter negatives, both from those in the present file and ones made in the future.

Strohmeyer and Carpenter, recognized as the foremost photographers of livestock in the United States and Canada, have played an important part in the advancement and improvement of the dairy industry. Recently, in honoring Mr. Strohmeyer, *The Ayrshire Digest* stated:

"Accurate, uniformly posed, and unretouched photographs of successive generations of dairy cattle are a primary aid in noting progress through breeding and identifying features which have proven common in animals of high production. Through the use of such photographs, breeders have been able to formulate ideals of dairy cattle type and more successfully achieve those ideals through selection of breeding stock."

The Van Dervort Studio, Cobleskill, New York, is situated in the dairy county of Schoharie, 40 mi. southwest of Albany. Operated by Clifford and Jane Van Devort since 1937, the studio offers a complete photographic service and is well-qualified to carry on the traditionally fine service furnished by Harry Strohmeyer and Charles Carpenter for many years.

## COMPLETED THESES

### Ph.D. degree

MILTON L. PEEPLES—forced convection heat transfer characteristics of fluid milk products. The Ohio State University, Columbus.

### M. S. degree

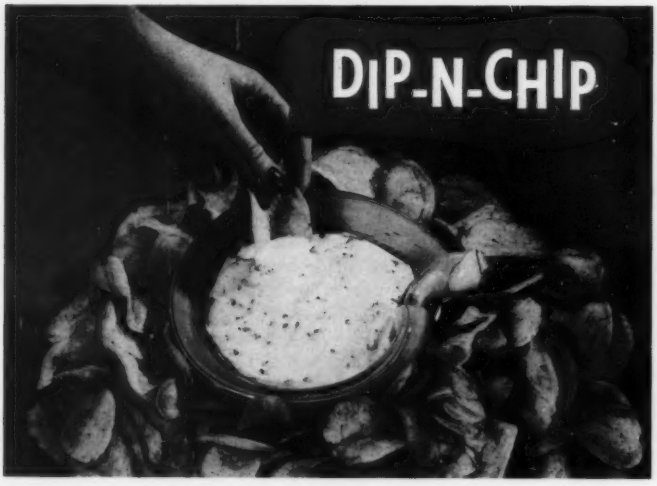
J. V. BRITTON, JR.—A study of the microbial flora of Cottage Cheese manufactured under plant conditions and stored at 5° C. University of Tennessee, Knoxville.

L. M. CARPENTER—A study of the provision of trace minerals for suckling calves. University of Tennessee, Knoxville.

D. G. EASTERLY—Removal of Strontium$^{89}$ and Calcium$^{45}$ from milk by use of ion-exchange resins. University of Tennessee, Knoxville.

A. F. MCFEE—The effects of various cooling methods on spermatozoan livability. University of Tennessee, Knoxville.

S. L. PARKINSON—A summary of the records on the Purebred Jersey dairy herd

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YOU'VE BEEN
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Millions of packages already sold to date

A COMPLETE PROGRAM AVAILABLE
FOUR FLAVORS
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Advertising Production Know-How Containers

YOU CAN BE IN THE DIP-N-CHIP BUSINESS PRACTICALLY OVER-NIGHT just use your present equipment—stir the flavor into your sour cream and dig in for profits.

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# FROM STARTER TO FINISH LACTOSE ASSURES COTTAGE CHEESE EXCELLENCE!

Lactose will give your cottage cheese an extra share of dairy freshness. It makes the dressing adhere to the curd... maintains the flavor appeal and appearance that create fast sales.

**LACTOSE IN COTTAGE CHEESE STARTERS** Gets them off to a brisk, dependable start every time. Insures uniform performance of cultures. Improves curd characteristics. Increases viability and activity of frozen cultures.

**LACTOSE IN COTTAGE CHEESE DRESSINGS** Extends shelf life of cottage cheese. Gives it a fresh, creamy appearance. Improves natural dairy flavor and reduces wheying off.

Only Western can supply Lactose in a full range of particle sizes. Strict chemical and bacteriological specifications, rigid quality control and years of experience assure top performance.

Take a good look at Lactose! For complete information, write Technical Service, Department 57E.

Lactose

**WESTERN CONDENSING COMPANY**

Appleton, Wisconsin

*World's Largest Producer of Lactose — Pure Milk Sugar*

IOSAN® Kills Mastitis Organisms

**IOSAN** is a patented germicidal cleaner that kills streptococcus, pseudomonas, E. Coli, staphylococcus and other organisms that cause and spread Mastitis. Its "Tamed-Iodine" killing power has been substantiated by laboratory tests that meet hospital standards. Iosan provides safe, low cost protection when washing udders and dipping teats.

**"Tattles" on milkstone.** Iosan quickly cleans and sanitizes bulk tanks and other equipment. It "tattles" on hard-to-remove or overlooked accumulations of milkstone with a tell-tale yellowish-brown stain that is easy to remove. Reduces bacteria counts to consistent lows, leaves equipment sparkling clean.

**Two-in-one product.** Iosan saves time and labor by replacing two or more single-action products. Also reduces hot water bills because it is used in tap or lukewarm water. For a free demonstration contact your regular supplier or Lazarus Laboratories Inc., Div. West Chemical Products Inc., 42-16 West St., Long Island City 1, N. Y.

Tamed Iodine
GERMICIDAL CLEANER

HOW TO MAKE THE WORLD'S BEST ICE CREAM

You must use the finest dairy ingredients, the finest flavors, the finest mix processing and freezing equipment, the finest packaging—*And The Finest Stabilizer*—KELCO STABILIZER.

**Dariloid XL®** *for your white mixes*
**Sherbelizer®** *for your chocolate mixes*

*Products of* **KELCO COMPANY**
75 Terminal Ave., Clark, N. J.
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530 W. Sixth St., Los Angeles 14, Calif.
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at the West Tennessee Experiment Station from 1928 to 1952. University of Tennessee, Knoxville.

J. D. SKEAN—Changes in milk proteins associated with the growth of psychrophilic bacteria. University of Tennessee, Knoxville.

M. SUBRAHMANYAM—Preservation of milk with hydrogen peroxide. University of Tennessee, Knoxville.

J. D. SULLIVAN—The effect of feeding L-thyroxine to dairy cows upon plasma protein-bound iodine and fecal thyroxine. University of Tennessee, Knoxville.

## DAIRY TECHNOLOGY SOCIETIES

**Atlanta** — EUGENE WELDEN, Supervisor, Conferences and Short Courses, Georgia Center for Continuing Education, University of Georgia, at the April 4 meeting discussed Continuing Your Education as an Adult. His speech stressed the importance of further education and what one can do to secure that education.

**Central Illinois**—April 13 meeting featured J. R. WRIGHT, Ambrosia Chocolate Company, Milwaukee, Wisconsin, speaking to the group's student dairy technology society and showing and illustrating the growth and harvest of cacao beans in Africa—Ghana, to be specific.

May meeting of the group is to feature The Netherlands, and the ladies are also invited.

**Central Michigan**—April 20 meeting was designated Sponsoring Membership Night, in honor of the group's many fine sponsors, who were presented their membership certificates. Guest speaker was GEORGE MCLEAN, secretary-treasurer of the United Dairy Workers, Local 83 of Detroit, speaking on the subject Labor Relations.

**Detroit**—T. I. Hedrick of the Department of Dairy of Michigan State University discussed at the April 11 meeting the topic of The Effect of Ultra High Pasteurization Temperatures on Various Fluid Dairy Products. New officers and committees for 1960 are: President, BRUCE BEVIER; vice-president, WAYNE MARKER; secretary, L. G. HARMON; treasurer, WENDELL BURGESS; sergeant-at-arms, DON MESELUS; board of directors, JOE MEISER, BILL PETERS, and BILL FORTH.

Program Committee, WAYNE MARKER (Chairman), BILL PETERS, MERRITT PETERSON, HAL FOSTER, JOHN TORREY. Membership Committee, MARION SHEPARD (Chairman), HART MORRIS, LEO SHEPARD, and W. BERNARDI. Reception Committee, BARNEY RICH (Chairman), TED DELCOMYN, JIM POLLARD, PETE PETROFF, and GENE BROWN. Nominating Committee, DON SMITH (Chairman), ANGELO DEFAZIO, ED-

WARD BROWN, DON STOWELL, and RUS MANY. Educational Committee, WILLIAM FORTH (Chairman), CLINTON SPRIGGS, and JOSE MEISER. The president and secretary are ex-officio members of all committees.

**Kansas City**—A social hour and dinner was held March 31 at the U-Smile Motel. This was the society's fifth program of the year. The guest speaker was SCOTT ANGEVINE, vice-president, Angevine's Laboratories, St. Louis, Missouri. His topic was: Cultures—Cottage Cheese and Quality. Announced purpose of this group is to render a service to all men and women employed in milk and milk products plants of that area, and to consider problems which will assist all of them in their efforts to furnish the consuming public with milk and milk products of high quality.

**Massachusetts**—D. H. JACOBSEN, Research Director, American Dairy Association, was the speaker at the March 7 meeting. He discussed the progress of the various research projects sponsored by the American Dairy Association. Annual election of officers was held at this meeting, with the following results: Newly elected Executive Committee Members: JERE SULLIVAN, New England Stabilizer Company; E. A. BLAKELOCK, Keiner-Williams Company; B. S. LEACH, Dairy Laboratory Service; JEREMIAH MCCARTHY, Boston Health Department; GREGORY MERCURIO, D'-Orsay Equipment Company; J. H. BRAGG, University of Massachusetts. Other Executive Committee Members: P. J. BLANCHARD, P. J. Blanchard & Sons; C. E. CHENEY, Eliot Creamery; D. J. HANKINSON, University of Massachusetts; C. A. HEDIN, American Dairy Association; K. F. ROLPH, K. F. Rolph Company; H. L. WILDASIN, H. P. Hood & Sons. Officers—1960-61: President—J. H. BRAGG, University of Massachusetts; Vice-President—C. A. HEDIN, American Dairy Association; Secretary—K. F. ROLPH, K. F. Rolph Company; Treasurer—C. H. LAWRENCE, Hubinger Company.

**Metropolitan**—A cocktail-dinner meeting was held April 12 at Gasner's Restaurant, New York City. Topic of the meeting was The Haunted Houses of Selling, and the speaker, D. R. OLSON, vice-president in charge of sales, Krim-Ko Corporation. New members of the organization are FRED NELSON, Economics Laboratory, Inc.; R. H. INGHAM, Creamery Package Manufacturing Company; and D. L. SPERONI, Sylvan Dairy, Inc.

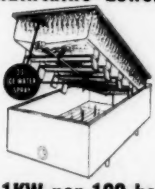
**North Carolina**—G. C. URIAN, Eastern Distributor, Grace Machinery Company, Philadelphia, Pennsylvania, was the speaker at the group's April 13 meeting, bringing members the latest information of mechanization of the Cottage Cheese process.


NO FROZEN MILK IN A

DARI-KOOL BULK MILK COOLER

* PREFERRED 3 TO 1 OVER ANY OTHER BRAND

● Cools Milk Faster and Maintains Lower Blend Temperatures Than Any Other Cooler



33° ice-water cooling removes milk heat fast. Does not lower butterfat test by freezing milk.

● Cools Milk for Less Than 1KW per 100 lbs. on a Yearly Average — and Under Full Load

Metered tests run at Young's Dairy, Twin Falls, Idaho, Dari-Kool used only .83 KW per 100 lbs. Send for this test.

● Protects Milk from Spoilage During Electric Power or Compressor Failures —

Dari-Kool's large reserve ice-bank protects milk from spoilage during such emergencies.

* In a national farm magazine preference survey of tank owners (all brands) Dari-Kool is now the 3 to 1 favorite over the second brand. Send for copy of survey.

DAIRY EQUIPMENT CO., Madison, Wis.

**Ohio**—The programs at the November meeting of the four Ohio Dairy Technology Societies included: Northeastern Ohio and Maumee Valley Dairy Technology Societies—What Is Dairy Technology?—a slide sequence, developed recently by the Department of Dairy Technology, depicting the story of Dairy Technology. Central Ohio Dairy Technology Society—Microorganisms as a Source of Food—presented by J. H. LICHTFIELD, Battelle Memorial Institute, Columbus. Cincinnati Dairy Technology Society—Selling Is Our Business—presented by H. "HAP" BOWSER, Dairy Pak Inc., Cleveland.

**Philadelphia**—April 14 meeting featured R. C. STANFILL, Director, Philadelphia Food and Drug Administration. His topic was: Report from the Food and Drug Administration. His talk covered the antibiotics residues and pesticides residues in dairy products, and the administrative problems involved.

**Tri-Cities**—The interesting topic of Off-Flavors in Milk and Their Identification was discussed by T. R. FREEMAN and WILLIAM LAGRANGE of the University of Kentucky's Dairy Department, using flavor samples to illustrate their talks.

**Western Michigan**—April 18 meeting featured a panel of guest speakers on the topic Dairy Product Quality Sales Through Retail Food Markets. Panel members included: MR. VAN PATTEN of the Dairy Division of the State Department of Agriculture; F. C. STRONG, sales manager for the Lockshore organization at Kalamazoo; L. V. EBERHARD, president of the Eberhard Food Stores, Grand Rapids; and PROFESSOR TROUT of the Dairy Department, Michigan State University. Products included in comments of panel members were: ice cream, butter, Cottage Cheese, cheese, milk, and cream.

**Animal Health Represenatives of Merck & Co.**

Twelve animal health sales representatives are shown above during a break in their spring training meeting at Merck Chemical Division, Rahway, New Jersey. For 2 wk. the men were brought up to date on marketing practices and programs, under a schedule supervised by J. J. Linney, assistant agricultural sales manager, and W. E. Guenther, sales specialist in merchandising at Merck.

Shown above, left to right, with residence addresses and sales territories are:

Front Row: D. J. Leyman, Fort Wayne, northern Indiana, western Michigan; C. D. Russell, Portland, Oregon, Idaho, Montana, and Washington; J. F. Clausen, Temple, Oklahoma, Texas Panhandle; S. R. Allen, Indianapolis, northern Indiana.

Second Row: R. M. Brady, Springdale, Arkansas, southern Missouri; J. R. Espy, St. Louis, eastern Missouri, southern Illinois; M. L. Horner, Kansas City, western Missouri, Kansas; T. D. Butler, Omaha, Nebraska and southwestern Iowa.

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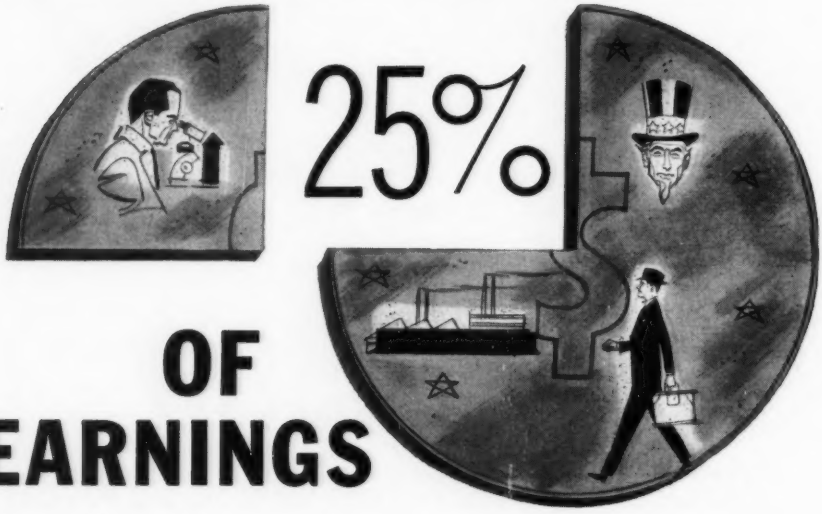

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# AUTOXIDATION OF MILK LIPIDS. I. IDENTIFICATION OF VOLATILE MONOCARBONYL COMPOUNDS FROM AUTOXIDIZED MILK FAT[1]

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## SUMMARY

The $C_1$ through $C_{10}$ n-alkanals, the $C_8$ through $C_{11}$ alk-2-enals, and acetone were conclusively identified as 2,4-dinitrophenylhydrazones, from the volatile material of milk fat oxidized to a TBA No. of 15 and a peroxide value of 14.3. In addition, presumptive evidence is presented for the identification of but-2-enal and the odd-numbered $C_5$ through $C_{15}$ n-alkan-2-ones. Approximately 63% of the isolated monocarbonyl compounds were n-alkanals and 34% alk-2-enals. The possible origin of the identified compounds, their role in the oxidized flavor of milk fat, and the relationship of the results of this paper to the findings of other workers are discussed.

---

That carbonyl compounds are major constituents in the oxidized flavor of dairy products is well established (10, 11, 15, 27). The types of flavors encountered appear to differ, depending upon the origin of the carbonyls in the milk lipids. According to Thurston, Brown, and Dustman (29), tallowy flavors arise from the glyceride fraction and the typical oxidized flavor from the phospholipid fraction. Published evidence on the types and amounts of oxidation products arising from the milk lipid fractions is not adequate, however, to substantiate the organoleptic observations. The most fruitful efforts have been directed to the identification of the carbonyl products of oxidized phospholipids. Forss, Pont, and Stark (10, 11) identified acetone, ethanal, n-hexanal, the $C_4$ to $C_{11}$ alk-2-enals, and the $C_6$ to $C_{11}$ alk-2,4-dienals from copper-induced, oxidized skimmilk. Although not specified, it is assumed that the origin of the bulk of these compounds was the unsaturated fatty acids of the phospholipids. More recently, Van Duin (5) has presented presumptive evidence for the $C_2$ to $C_{18}$ alkanals, $C_5$ to $C_{11}$ alk-2-enals, $C_8$ and $C_9$ alk-2,4-dienals, and a number of ketones in oxidized phospholipids from butter.

Information on the identity of the carbonyl products of oxidized milk fat is not as complete as that for the phospholipids. Keeney and Doan (15), in a study of oxidized fat, found 2-hydroxypropanal; unsaturated unconjugated and unsaturated conjugated ketones with the carbonyl oxygen on some other than the No. 2 carbon. More recently, Tamsma (27) oxidized milk fat at 100° C. and separated the volatile carbonyl compounds into three fractions characterized as unconjugated unsaturated, conjugated monene, and conjugated diene.

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[1] Technical Paper No. 1267, Oregon Agricultural Experiment Station.

The research reported herein was designed to further characterize the volatile carbonyl compounds that comprise the oxidized flavor defect of milk fat. Conclusive evidence is presented for the identification of the $C_1$ through $C_{10}$ n-alkanals and the $C_5$ through $C_{11}$ alk-2-enals from milk fat oxidized by exposure to air and diffuse light at room temperature. Presumptive evidence for but-2-enal and traces of the odd-numbered $C_5$ through $C_{15}$ n-alkan-2-ones also is included. The qualitative and quantitative data revealed that the types and amounts of carbonyl compounds predominating in the oxidized triglyceride fraction of milk lipids are indeed different from those reported to arise from the oxidation of milk phospholipids.

## MATERIALS

*Milk fat.* Milk fat samples were prepared from fresh pasteurized cream (70° C., 30 min.) by churning, melting the butter at 55° C., washing four times with water at 55° C., and removing the water with a small milk separator. Precautions were taken to avoid contamination of the fat with catalytic metals during the preparation steps. Aliquots of the clear oils were placed in tightly stoppered flasks and stored in the dark at −20° C. Aliquots were taken from storage as needed and allowed to oxidize by exposure to air at 20 to 25° C. in diffuse light. Fresh samples for control studies were prepared from fresh cream when needed. Negative phosphorus tests on the fat samples indicated that the method for preparation of the fat was adequate for removal of phospholipids.

*Solvents.* Hexane was treated as described by Day, Bassette, and Keeney (4). Low-boiling petroleum ether (37-40° C.) was treated the same way. Chloroform and ethanol were redistilled. The trace amounts of carbonyl contaminants remaining in the solvents were accounted for by treating the solvents with 2,4-dinitrophenylhydrazine[2] and isolating the derivatives which were then purified by column chromatography (4). Minute quantities of methanal and ethanal were found.

*Authentic carbonyl compounds.* The n-alkanals and alkan-2-ones were obtained commercially, except for hexanal and 2-pentadecanone. Hexanal was prepared by dry distillation of the calcium salt of caproic and formic acids, and 2-pentadecanone was prepared by dry distillation of the calcium salt of myristic and acetic acids, as described by Richter (23). The alk-2-enals from $C_5$ through $C_{11}$ were synthesized by the procedure of Radlove (22), with the following modifications: Attempts to distill the intermediates, i.e., the diethyl acetal of 2-bromoalkanals, were not successful, due to excessive frothing in the distillation pot. Therefore, the syntheses were carried to completion without purification of intermediate compounds and the crude alk-2-enals were removed from the dark-brown reaction mixtures by steam distillation. The crude alk-2-enals were extracted from the steam distillates, and the ether removed to give clear to slight yellow-colored oils. A portion of each crude alk-2-enal thus formed was reacted with 2,4-dinitrophenylhydrazine and the derivative was subsequently purified by column chromatography.

[2] 2,4-dinitrophenyl is hereinafter abbreviated DNP.

The DNP-hydrazones of all authentic carbonyl compounds were prepared by reaction with 2 *N* HCl, saturated with DNP-hydrazine. The DNP-hydrazones were purified by column chromatography, crystallized from low-boiling petroleum ether, and melting points determined.

## METHODS

*Iodine absorption number.* Hanus method (2).

*Peroxide value.* Peroxides were determined by the A.O.C.S. method (1) and are expressed as milliequivalents of peroxide per kilogram of fat.

*2-Thiobarbituric acid number (TBA No.).* The procedure of Sinnhuber and Yu (25) was employed and the TBA number refers to the milligrams of malonaldehyde per kilogram of fat. By conversion of the optical density readings of Dunkley and Jennings (6), with the assumption that the milks analyzed by them contained 4% fat, milks with slight oxidized flavors should exhibit a TBA number of 0.072.

*Determination of phosphorus in milk fat.* The fat was ashed as described by Pont (20) and tested for phosphorus by the method of Fiske and Subbarrow (8).

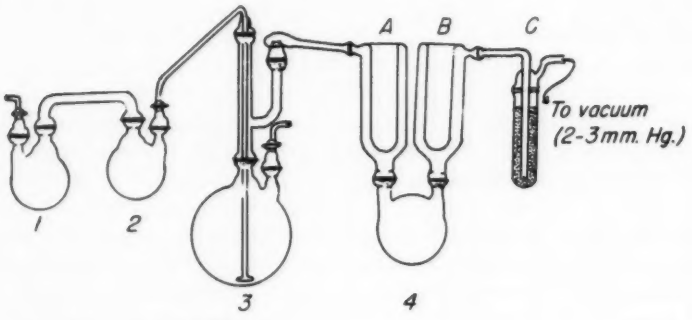
*Isolation of volatile monocarbonyl compounds.* The carbonyl compounds were isolated from the fat by means of the steam deodorization apparatus illustrated in Figure 1. This apparatus is a modification of the one described by Patton and Tharp (19). Five hundred-gram batches of fat were warmed to 40° C. at 2-3 mm.Hg pressure and stripped with steam generated at the same pressure. Each distillation was terminated when approximately 250 ml. of water had passed from the steam generator. These mild conditions, while not as efficient as higher temperatures, were used in order to keep heat-generated compounds to a minimum.

Upon completion of a distillation, 250 ml. of 5 *N* HCl saturated with DNP-hydrazine was added to Flask No. 4 and the frozen distillate was allowed to melt and drain into the reagent. The cold finger traps were finally washed with a total of 100 ml. of warm ethanol to remove any material adhering to the glass surface. That portion of the distillate contained in Trap C was removed by washing with 50 ml. of ethanol and this material was combined with that in Flask No. 4, giving a final volume of 650 ml. of reaction mixture. Flask No. 4 was then closed with glass stoppers and allowed to stand 12 hr. The DNP-hydrazones of the monocarbonyl compounds were obtained from the reaction mixture by extracting five times with 100-ml. portions of hexane. The extracts were combined and the hexane removed at reduced pressure to yield a dry residue which was designated the monocarbonyl mixture.

That the hexane extraction procedure was adequate for isolation of the bulk of the DNP-hydrazones of monocarbonyls from the reaction mixture was demonstrated by the following experiment: After hexane extraction, the reaction mixture was extracted three times with 100-ml. portions of chloroform; the extracts were combined and the chloroform removed to yield a dark-red residue. The dark-red residue (chloroform extract) was exhaustively extracted with hexane; the hexane extract was concentrated and analyzed by column chromatography (4). Trace amounts of $C_1$ to $C_3$ monocarbonyl derivatives were found.

Reaction of a portion of the chloroform extract with alcoholic KOH yielded a deep blue to violet color indicative of *bis*-DNP-hydrazones. For all practical purposes, this mixture was considered to contain no significant amounts of monocarbonyl compounds and was set aside for future investigation.

*Column and paper chromatography.* The nitromethane-hexane-Celite column described by Day *et al.* (4) was used as the major tool for separation and purifica-




1. Steam generator (500 ml)
2. Steam trap ( " )
3. Steam-stripping flask (2 liter); warmed to 40°C.
4. Reaction flask (1 liter)
A & B. Cold finger traps (powdered dry ice)
C. Glass bead trap ( " )

FIG. 1. Apparatus for isolation of volatile carbonyl compounds from milk fat.

tion of the monocarbonyl mixtures. This procedure was complemented by the paper chromatography methods of Huelin (13) and Klein and de Jong (16).

*Spectrophotometric analyses.* Absorption spectra of the DNP-hydrazones dissolved in chloroform were obtained with the Beckman Model DU and the Beckman DK-I spectrophotometers over the range of 290 to 450 mμ.

*Melting point analyses.* Melting points were taken on a Kofler micro-hot stage and were recorded as corrected melting points. Samples for melting points were prepared by placing several crystals on the glass slide, then by covering the crystals with a cover slip and firmly placing the eraser end of a pencil on the cover slip and rotating the pencil a homogenous, powdery mixture of crystals resulted. This procedure was especially useful for preparing samples for mixture melting points.

## RESULTS

*Qualitative analyses.* Fresh milk fat with an iodine number of 31.1, and with no detectable amount of phosphorus, was oxidized to a peroxide value of 14.3 and a TBA number of 15.0. The DNP-hydrazone mixture of the monocarbonyl compounds, obtained from 1,500 g. of this fat, was separated into 11 bands on

20-g. nitromethane-hexane-Celite columns. A number of columns was required to handle the entire monocarbonyl mixture and specific bands were pooled for subsequent analysis. A column showing separation of the mixture into the various bands is depicted in Figure 2. The absorption maxima of the initially separated bands are given in Table 1. These data suggested that Bands 1, 3, 5, 7, and 9 were composed of ketones, Band 6 and 10 of saturated aldehydes, and Bands 2, 4, and 8 of alk-2-enals. Subsequent melting point analysis of crystals representing each band revealed, however, that in most cases the bands were composed of multiple-component mixtures. This was further substantiated in the cases of Bands 3, 5,

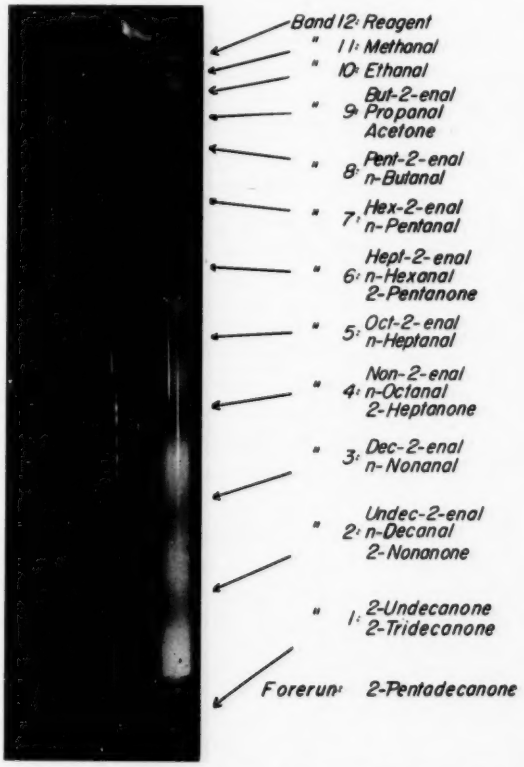



FIG. 2. Nitromethane-hexane-Celite partition column showing initial separation of the DNP-hydrazone mixture of the volatile monocarbonyl compounds from oxidized milk fat.

and 7 which, although appearing to be ketones according to absorption maxima, gave odors of aldehydes when regenerated by the sulfuric acid method of Bassette and Day (3).

TABLE 1

Identification data for the 2,4-dinitrophenylhydrazones of volatile monocarbonyl compounds from autoxidized milk fat

| Band No. | λMax. | Fraction | DNP-hydrazone | T. V.[a] Unknown | T. V.[a] Authentic | λMax. (mμ) Unknown | λMax. (mμ) Authentic | M P° C. Unknown | M P° C. Authentic | M P° C. Mixture |
|---|---|---|---|---|---|---|---|---|---|---|
| Forerun | 363 | | 2-Pentadecanone | 4.0(A)[b] | 4.0 | 363 | 363 | | 75–76 | |
| 1 | 363 | 1A | 2–Tridecanone | 5.0(A) | 5.0 | 363 | 363 | | 69–71 | |
| | | 1B | 2-Undecanone | 7.3(A) | 7.3 | 364 | 364 | | 63–64 | |
| | | 2A | 2-Nonanone | | 8.2 | 360 | 363 | | 54.5 | |
| 2 | 372 | 2B | n-Decanal | 8.3(A) | 8.3 | 358 | 358 | 104 | 106 | 104–106 |
| | | 2C | Undec-2-enal | 8.3(A) | 8.3 | 373 | 373 | 122–123 | 120–121 | 121–123 |
| 3 | 364 | 3A | n-Nonanal | 9.3(A) | 9.3 | 358 | 358 | 105–106 | 107 | 104–106 |
| | | 3B | Dec-2-enal | 9.3(A) | 9.3 | 373 | 373 | 126–128 | 128–129 | 126–128 |
| | | 4A | 2-Heptanone | | 12.0 | 361 | 363 | | 72 | |
| 4 | 371 | 4B | n-Octanal | 12.7(A) | 12.5 | 358 | 358 | 104–106 | 106 | 103–106 |
| | | 4C | Non-2-enal | 13.0(A) | 13.5 | 373 | 373 | 125–126 | 124.5–126 | 124–126 |
| 5 | 363 | 5A | n-Heptanal | 16.2(A) | 16.1 | 358 | 358 | 107–108 | 107–108 | 107–108 |
| | | 5B | Oct-2-enal | 17.0(A) | 17.2 | 373 | 373 | 126–129 | 128.5–129 | 127–128 |
| | | 6A | 2-Pentanone | | 20.0 | 363 | 363 | | 144 | |
| 6 | 359 | 6B | n-Hexanal | 20.5(A) | 21.0 | 358 | 358 | 108–109 | 108–109 | 108–109 |
| | | 6C | Hept-2-enal | 22.6(A) | 22.2 | 372 | 373 | 128–129 | 127–128 | 127–129 |
| 7 | 361 | 7A | n-Pentanal | 24.0(A) | 24.0 | 358 | 358 | 104–106 | 106.5–107.5 | 104–106 |
| | | 7B | Hex-2-enal | 24.0(A) | 24.0 | 373 | 373 | 141–142 | 143–144 | 141–143 |
| 8 | 368 | 8A | n-Butanal | 35.0(B) | 35.0 | 358 | 358 | 122 | 123 | 121–123 |
| | | 8B | Pent-2-enal[c] | 36.0(B) | 36.0 | 373 | 373 | 154–156 | 150–154 | 150–154 |
| | | 9A | Acetone | 42.0(B) | 42.0 | 362 | 363 | 126–127 | 126 | 125–126 |
| 9 | 362 | 9B | Propanal | 42.0(B) | 42.0 | 357 | 358 | 153 | 156 | 152–154 |
| | | 9C | But-2-enal | 48.0(B) | 47.0 | 370 | 373 | 170–176 | 190 | 170–175 |
| 10 | 356 | | Ethanal | 70.0(B) | 70.0 | 355 | 356 | 148 | 166 | 164–166 |
| 11 | 348 | | Methanal | Rf values agreed | | 348 | 348 | 163 | 166 | 162–165 |

[a] T. V. = Threshold volume in milliliters [see Reference (4)].

[b] Indicates the types of partition column used [see Reference (4)].

[c] The authentic pent-2-enal could not be purified because of contamination with traces of an isomer.

Day *et al.* (4) reported that the nitromethane-hexane-Celite partition column would not separate mixtures containing alkan-2-ones, alkanals, and alk-2-enals with chain lengths of $C_n$, $C_{n+1}$, and $C_{n+2}$, respectively. Results in our laboratory show that alk-2,4-dienals with chain lengths of $C_{n+4}$ also can occur in these mixtures. Each of the initial 11 bands, therefore, could contain from one to four, or possibly more, components such as described. In this investigation, it was observed that when chromatographing an individual multiple component band, purposely overloading the columns caused the components to become more concentrated in specific sections of a band area. This made it possible to obtain pure compounds by fractionation of each multiple component band into as many as ten fractions, obtaining absorption spectra of the fractions, pooling fractions with similar absorption maxima, and rechromatographing pooled fractions until pure compounds were obtained. For example, Band 3 of Table 1 was initially separated into seven fractions, giving the following absorption maxima: Fraction 1 = 358 mμ; 2 = 359 mμ; 3 = 363 mμ; 4 = 365 mμ; 5 = 369 mμ; 6 = 371 mμ; 7 = 373 mμ. Fractions 1 and 7 were pure n-nonanal and dec-2-enal, respectively. Fractions 2, 3, and 4 were pooled as were 5 and 6, and subsequently rechromatographed. It was possible, by this procedure, to build up sufficient quantities of pure compounds for identification.

Following purification by column chromatography, the eluates were taken to dryness under reduced pressure and the residues crystallized from petroleum ether. The crystals were then transferred to filter paper with a micro-eye dropper, dried, and used for identification studies. Identification of the DNP-hydrazones was dependent upon the following data. Chain lengths were ascertained by column (4) and paper chromatography (13, 16). Conjugated unsaturation and functional groups were revealed by absorption spectrophotometry. Agreement between the melting points of authentic and unknown derivatives, coupled with the absence of depressions in mixture melting points, served as the criterion for conclusive identification.

Evidence for the conclusive identification of 18 monocarbonyl compounds isolated from autoxidized milk fat is presented in Table 1. Although acetone was the only ketone conclusively identified, presumptive evidence for the presence of odd-numbered $C_5$ to $C_{15}$ alkan-2-ones was obtained. Fractions 2A, 4A, and 6A of Table 1 were detected spectrophotometrically in the front of their respective bands. These fractions were not readily visible on the chromatographic column, but were found at very low concentrations in eluates immediately preceding the front edge of the bands. For example, if it were possible to divide Band 2 on the basis of absorption spectra, one would first see an absorption maxima of 360 mμ at the edge of the band, which then drops to 358 mμ and again raises to 373 mμ as one progresses towards the tail of the band. Insufficient quantities of Fractions 2A, 4A, and 6A prevented conclusive identification. Paper chromatograms, however, gave $R_f$ values congruent with the authentic ketone derivatives. Odors of these fractions when regenerated were neither typical of aldehydes nor of ketones but were suggestive of mixtures.

The evidence given in Table 1 suggests that the forerun and Fractions 1A and 1B are ketones. The $R_f$ values by the Klein and de Jong method (16) were in agreement with those of the respective authentic derivatives. Regeneration of the free carbonyl compounds gave odors typical of ketones. The oily, amorphous crystals obtained from these fractions gave no distinct melting points.

Repeated attempts to purify Fraction 9C (Table 1) by column and paper chromatography were unsuccessful. The highest melting range obtained for the dark-red crystals was 170–176° C. The $R_f$ value of this material, by the Huelin method (13), was identical to the authentic DNP-hydrazone of but-2-enal.

Tamsma (27) obtained evidence for conjugated alk-2,4-dienals in oxidized milk fat, and several of these compounds were identified by Forss *et al.* (10, 11) in copper-induced, oxidized skimmilk. Their conspicuous absence in our volatile monocarbonyl mixture suggested possible destruction during the many manipulations inherent in identification studies as reported by Nonaka *et al.* (18). To clarify the point, the DNP-hydrazones of monocarbonyl mixtures from three fat samples, oxidized to peroxide values of 0, 4.3, and 15.0, and TBA numbers of 0.48, 5.2, and 15.8, were separated into the 11 major chromatographic bands. Individual bands were then separated into subfractions and absorption spectra obtained. The analyses were carried to completion within 48 hr. and precautions were taken to minimize oxidative effects. Again, no evidence of the presence of alk-2,4-dienals was obtained.

*Quantitative analysis.* The efficiency of the steam stripping procedure for removal of carbonyl compounds from fat was determined by adding weighed amounts of undec-2-enal, n-nonanal, n-heptanal, and n-pentanal to 500 g. of fresh milk fat and measuring the quantities recovered as DNP-hydrazones by spectrophotometric procedures. The recoveries were as follows: undec-2-enal 56%; n-nonanal 75%; n-heptanal 63%; and n-pentanal 61%. The true efficiency of the steam stripping method was found to be higher than the above values when the per cent reaction of the four compounds with DNP-hydrazine was taken into account. The per cent reaction of the above four compounds was 94, 93, 76, and 87%, respectively. This would raise the true efficiency of the steam stripping method for the four compounds to 60, 81, 83, and 70%. The reaction conditions were identical to those used to isolate the carbonyls from distillates of oxidized fat.

Table 2 shows the quantities of free volatile carbonyls occurring in milk fat oxidized to a TBA number of 15.0 and a peroxide value of 14.3. These data were obtained by separating the DNP-hydrazone mixture into the fractions shown in Table 2, measuring the absorbance of each fraction at its wave length maximum, and then calculating the quantity of carbonyls represented thereby. Inherent losses in purifying each fraction precluded quantitative measurements of every compound identified in the respective fractions. The quantity of carbonyls represented by a particular fraction, therefore, is expressed in terms of the major compound contained in that fraction. The major compounds representing the various fractions for the quantitative measurements are included in Table 2.

*Control studies.* The monocarbonyl compounds present in 500 g. of fresh fat were isolated and handled in the same manner as described above. Initial column

chromatography of the crude DNP-hydrazone mixture gave only bands for acetone, methanal, and ethanal, but the forerun contained traces of derivatives. Therefore, the forerun was pooled, concentrated, and rechromatographed to give a total of seven bands. These bands exhibited threshold volumes (4) and $R_f$ values identical to the respective bands from oxidized fat. No evidence for Band 7 was

TABLE 2

Quantitative analyses of carbonyl compounds from oxidized and fresh milk fat

| Column Band No. | Calculated as | $\mu M \times 10^4$ | Mg. of carbonyl/kg oxidized fat | $\mu M$ of carbonyl/kg oxidized fat | Mg. of carbonyl/kg fresh fat | $\mu M$ of carbonyl/kg fresh fat |
|---|---|---|---|---|---|---|
| 1 | 2-Undecanone | 2.3 | 1.8 | 10.6 | 0.13 | 0.76 |
| 2 | Undec-2-enal | 2.7 | 5.9 | 35.0 | 0.18 | 1.7 |
| 3 | n-Nonanal | 2.3 | 2.5 | 17.8 | 0.07 | 0.50 |
| 4 | Non-2-enal | 2.7 | 14.2 | 103.0 | 0.06 | 0.43 |
| 5 | n-Heptanal | 2.3 | 15.3 | 134.0 | 0.16 | 1.4 |
| 6B | n-Hexanal | 2.3 | 15.6 | 156.0 | 0.14[a] | 1.4 |
| 6C | Hept-2-enal | 2.7 | 5.4 | 48.0 | ..... | ..... |
| 7 | n-Pentanal | 2.3 | 13.8 | 160.0 | ..... | ..... |
| 8 | Pent-2-enal | 2.7 | 3.4 | 40.0 | 0.07 | 0.87 |
| 9B | Propanal | 2.3 | 5.2 | 90.0 | 0.07[b] | 1.2 |
| 9 | But-2-enal | 2.7 | 0.7 | 10.0 | ..... | ..... |
| 10 | Ethanal | 2.3 | 1.4 | 32.0 | 0.7 | 16.0 |
| 11 | Methanal | 1.9 | 0.4 | 13.0 | ..... | ..... |
| | Total | | 85.6 | 849.4 | 1.58 | 24.3 |

[a]Band 6 Calculated as n-hexanal.
[b]Band 9 Calculated as propanal.

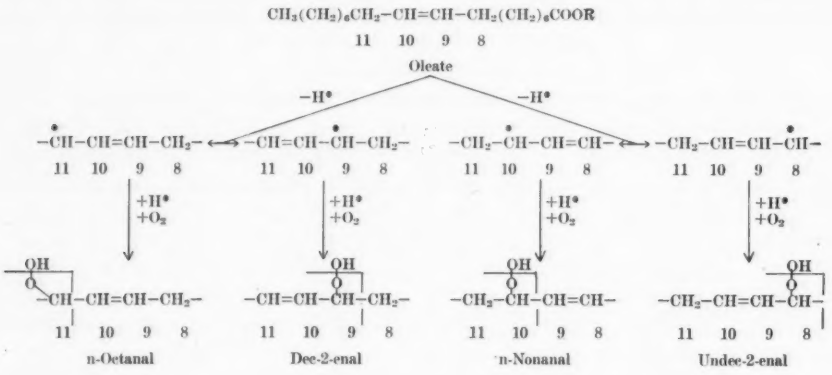
obtained. Identical results were obtained in a second experiment, wherein a different fat sample was used. Quantitative data were obtained and are presented in Table 2. Regeneration of the DNP-hydrazones representing each band and observations of resulting odors gave leads to the types of compounds present in several of the bands. Bands 3, 4, 5, and 6 exhibited odors suggestive of aldehydes. Because of insufficient quantities of DNP-hydrazones, odors of the carbonyls regenerated from Bands 1, 2, 8, 9, 10, and 11 were too faint for characterization.

*Characterization of the odors of identified carbonyl compounds.* Observations on the odors of authentic carbonyls, both individually and in mixtures, and of the regenerated DNP-hydrazones of carbonyls identified from oxidized milk fats, revealed that the monocarbonyl mixture isolated in this study closely resembles the oxidized odor of milk fat. It was also noted that the identified compounds were classifiable into definite odor categories. The $C_7$ to $C_{11}$ alk-2-enals exhibited metallic, cardboard type odors, whereas the $C_7$ to $C_{10}$ n-alkanals gave oily-tallowy odors. The $C_5$ and $C_6$ alkanals and alk-2-enals gave fruity type odors, and the odor of the shorter-chain monocarbonyls was more sharp and irritating.

## DISCUSSION

Origins for some of the carbonyl compounds identified in this study are explainable by the oxidation theories of Farmer and co-workers (7). Reaction schemes have been published for the production of ethanal, propanal, hexanal,

but-2-enal, and pent-2-enal from linoleate (14), and hept-2-enal and oct-2-enal from linolenate (24, 26). A similar scheme for the oxidative degradation of oleate which may account for octanal, nonanal, dec-2-enal, and undec-2-enal follows:




Neither the above nor similar reaction schemes explain the presence of n-pentanal, n-heptanal, hex-2-enal, and non-2-enal, unless it is assumed that unusual migrations of double bonds occur. It is notable that in this study, pentanal, heptanal, and non-2-enal account for over 50% of the total volatile carbonyls isolated from oxidized milk fat.

According to the early work of Tauffel *et al.* (28), followed by the recent findings of Patton and Tharp (19), alkan-2-ones can be generated from fats through the action of heat and water. The same phenomena may account for the trace quantities of odd-numbered $C_5$ through $C_{15}$ ketones observed in this investigation. The increase in quantity of Band 1 (Table 2) in oxidized fats over that of fresh fat can not be explained by heat generation. The evidence obtained for the identity of compounds contained in Band 1, although suggestive of ketones, does not preclude the presence of aldehydes in the same fraction.

The presence of a large number of carbonyls in fresh fat samples, some of which appear to be aldehydes, suggests that lipid oxidation may have occurred in the creams used as the source of fat. According to the work of Hills and Thiel (12), oxidation of milk lipids may commence shortly after the milk leaves the cow's udder.

Some of the results presented in this paper are contrary to the findings of other workers (15, 27). Keeney and Doan (15) concluded that the bulk of the volatile carbonyl compounds from oxidized milk fat were ketones, with the carbonyl oxygen in a position other than the No. 2 carbon. Their conclusions were based on the specificity of the Girard reagent for separation of ketones from aldehydes, a negative iodoform test, elemental analysis, and absorption spectra. The Girard reagent is nonspecific (9), negative iodoform tests do not preclude aldehydes, elemental analyses will fit both aldehydes and ketones of comparable chain length,

and absorption spectra can be misleading, as shown in the second column in Table 1 of this paper. We suggest, therefore, that Fraction 1 and combined Fractions 2, 3, and 4 of Keeney and Doan (15) are similar to Band 6 and Band 4, respectively, in Table 1.

Contrary to the results of Tamsma (27), we found the conjugated monene carbonyl fractions (34% of the total volatile carbonyls) to be significant in the oxidized odor of the milk fats investigated. Furthermore, we found no evidence that unsaturated unconjugated carbonyl compounds were the major components of the oxidized fat odor, as reported by Tamsma. Rather, it would appear that his unconjugated fraction was similar to the alkanals identified in this study. It immediately becomes apparent that while the evidence presented herein does not reveal unsaturated, unconjugated carbonyls, it does not preclude their existence. Shifts in double bonds from unconjugated to conjugated positions could occur without detection during manipulation of the fat, the isolation of volatiles, and the formation and purification of derivatives. Evidence to refute such a possibility would include: (1) the results presented above, in which the mixture of identified alkanals and alk-2-enals gave an odor closely resembling that of oxidized milk fat; (2) the generally accepted concepts for the mechanism(s) of fat oxidation; (3) studies of hydroperoxides isolated from oxidized unsaturated fatty acids in which *cis-trans* conjugated monomeric monohydroperoxides were the major initial oxidation products (17, 21). Tamsma (27) also reported the presence of conjugated diene carbonyls in oxidized milk fat which were not found in our samples. In this case, the differences in results may be due to differences in the composition of milk fat samples and oxidation conditions employed for the two studies.

In view of the large number of aldehydes identified from oxidized milk fat, it does not appear feasible to attribute the odor defect to a few specific compounds. Rather, the presence of the complete spectrum of compounds seems necessary for the typical odor associated with oxidized milk fat. Of the carbonyls isolated from oxidized milk fat, 63% were alkanals and 34% were alk-2-enals, whereas the majority of the carbonyl compounds reported by Forss *et al.* (10, 11), in oxidized phospholipids in skimmilk, were alk-2-enals and alk-2,4-dienals. Therefore, the differences in the types of compounds appearing to predominate in oxidized glyceride and phospholipid fractions may well explain the distinctly different oxidized odors associated with the two fractions.

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# CHEMICAL COMPOSITION OF MILK [1]

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**SUMMARY**

Data are presented on analytical determinations of 23 milk components in 258 bulk samples. The significance of seasonal and area differences in these components was measured. All components, except proteose-peptone nitrogen, nonprotein nitrogen, soluble calcium, and insoluble organic phosphorus, show significant variations with season. Statistical evidence of a greater significance in seasonal changes in citric acid, magnesium, and the soluble forms of phosphorus may implicate these in seasonal difficulties encountered in milk processing.

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Interest in the chemical composition of milk and its variations has increased in recent years. Recent reviews (1, 2, 10) indicate the voluminous literature on the subject. It has become increasingly clear that variations in composition influence the stability of milk during the processing of concentrated and sterilized products or result in processing difficulties or defects in cheese, ice cream, market cream, and other dairy products. Changes in processing modifications may reduce or prevent some of these defects, but it is still a matter of conjecture which of the changes in milk are the primary causes.

Many factors are known to influence milk composition, such as cattle breed, stage of lactation, environment, and feeding and management practices. However, data in the literature are insufficient for prediction of the variation in composition that could be expected from different bulk milk supplies and how much variation might occur in a given milk supply during changes in the seasons. Data are also lacking on the relationship of the milk constituents to each other.

This is a report of a project to determine the chemical composition of several milk supplies over a long period, in order to measure the magnitude of changes in selected milk constituents. The interrelationship of these constituents will be reported in a subsequent paper.

The milk samples were received at biweekly intervals from six processing plants in the principal milk-producing areas of California: Visalia, Newman, Davis, and Willows, representing the southern to northern sections of the hot, dry central valleys; and Petaluma and Fernbridge, representative of the cool, moist coastal regions. These areas also exemplify differences in feeding and management practices, as well as breed differences. Visalia is an area of predominantly Holstein herds. There are increasing numbers of other breeds up the valley. In the coastal areas, Jersey and Guernseys are found in greater numbers. All samples received from these areas were considered to be representative of the bulk manufacturing milk supply of the cooperating creamery.

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The milk was cooled in ice water and then shipped in a chilled stainless steel thermos by bus, so that it arrived for analysis the same day. Any samples in unsatisfactory condition on arrival were discarded. Data from 258 milk samples received during the period October, 1956, to June, 1958, were used in preparing this paper. Many more samples were received and partially analyzed, but statistical analysis requires exclusion of samples for which data on some constituents are lacking.

Estimates were made of 23 components of milk: 1. Total solids by Monjonnier; 2. Fat by Babcock; 3. Solids-not-fat by difference. 4. The total nitrogen by semimicro-Kjeldahl was distributed as follows: 5. Casein N; 6. Heat-denaturable whey protein; 7. Proteose-peptone N, and 8. Nonprotein N by Rowland's method (9).

Phosphorus was determined by a modification of the Harris-Popat method (5). Digestion was accomplished with Kjeldahl reagent. With samples that did not require digestion, 0.4 ml. Kjeldahl reagent was added to adjust acidity to about pH 1, the level necessary for the conditions of the reaction. The general procedure was to add to a 25-ml. volumetric flash, 5-ml. sample (containing 0.025–0.125 mg. phosphorus), dilute to about 15 ml. with water, add 0.4 ml. Kjeldahl reagent and 1.0 ml. of 5% ammonium molybdate solution, and mix thoroughly. Then 2.0 ml. of elon solution was added and the mixture diluted to volume. The optical density of each sample was read at 820 mμ against a blank 15 min. after the elon addition, and the phosphorus was estimated from a standard curve in the usual way.

The phosphorus distribution scheme was based on the methods of Graham and Kay (4) and Pyne (8). Four direct analyses were made, as follows: 9. Total phosphorus, determined on digestion of whole milk; 10. Total soluble phosphorus, determined on digestion of rennet whey; 11. Total inorganic phosphorus, determined on the trichloroacetic acid (TCA) filtrate from milk, which is the same as Rowland's C filtrate for determination of nonprotein nitrogen; and 12. Soluble inorganic phsophorus, determined on TCA filtrate of rennet whey.

From these analyses, the following forms of phosphorus were calculated: 13. Organic phosphorus = 9 − 11; 14. Soluble organic phosphorus = 10 − 12; 15. Insoluble organic phosphorus = 13 − 14; 16. Insoluble inorganic phosphorus (colloidal) = 11 − 12; 17. Total insoluble phosphorus = 9 − 10.

18. Citric acid content of milk was estimated by a pentabromoacetone procedure based on the methods of Perlman *et al.* (7) and Ettinger *et al.* (3). A 1-ml. sample containing 0.1–0.5 mg. citric acid, and 11 ml. 15% TCA were added to a 50-ml. glass-stoppered flask. One milliliter of 1 *M* bromide-bromate solution was added while shaken thoroughly, and 2 ml. of 0.125 *M* $KMnO_4$ was then added at a very slow rate and with vigorous agitation. The mixture was allowed to stand at room temperature for 10 min. Sufficient 2 *M* hydrazine solution (about 7 drops) was added to decolorize the solution completely. The stopper and sides of flask were washed with 1–2 ml. water, a few glass beads were added, and the solution was extracted with 15 ml. petroleum ether (washed with conc. $H_2SO_4$) by shaking vigorously for 2 min. The flask was allowed to stand until the layers

separated. Then, to tubes containing 5 ml. 50% pyridene-water mixture and 5 ml. 4% NaS solution, 10 ml. of the petroleum ether extract was added, and the tubes were shaken vigorously for 1 min. The yellow color developed within 5 min. and was stable for 45 min. Optical density was measured at 450 mμ, and citric acid content was estimated from a standard curve.

The calcium and magnesium contents were measured by the procedure suggested by Jenness (6). 19. Total calcium and magnesium (expressed as milligrams of calcium); 20. Total calcium, and 22. Soluble calcium (the form in rennet whey). 21. Total magnesium = (19 − 20) × 24.3/40, and 23. Insoluble calcium = 20 − 22.

Much of the statistical analysis (means, standard deviations, correlations) was calculated at the University Computer Center, using punch cards and a 701 computer. Other portions of the analysis were made according to standard statistical procedures, i.e., analysis of variance, significance of differences.

The mean compositions of the milks from the different areas over the 21-mo. test period are shown in Table 1. The range of variation to include 95% of the cases can be readily calculated from mean ± 2 standard deviations. It is apparent that most of the major constituents vary at least 10–20%. The area differences reflect variations, primarily due to breed but also undoubtedly influenced by climate and management and feeding practices. The parts of the variation due to season and to area were measured by analysis of variance, but no attempt was made to study the effects of management or feeding, except where these factors entered into area differences.

The milks from the various areas differed significantly in their content of many constituents (Table 2). The means were ranked in increasing order and the difference between them was measured for significance for use of the appropriate formula for the least significant difference, which is based upon Student's "t" distribution. The areas underscored by the same line indicate that the milks contained similar levels of the constituent measured. Any areas not underscored by the same line are significantly different in the constituents. The milks showed significant variations by areas for all constituents except soluble organic phosphorus, citric acid, and magnesium.

In a similar fashion, differences due to season (Table 3) were tested for significance. In Table 4, the seasons not underscored by the same line were significantly different in the constituent measured. In this case, the quantities of proteose-peptone nitrogen, nonprotein nitrogen, organic phosphorus, insoluble organic phosphorus, and soluble calcium did not show significant variations with season. All the other constituents did vary significantly with season.

It can be seen from the data in Table 3 that most constituents were at their lowest levels in May, June, and July, and at their highest levels in November, December, and January. Variation from sample to sample was considerable, however, even though they were from bulk milk supplies. This has been found by other workers, too.

TABLE 1

Composition of milk from six areas of California

| | Visalia | | Newman | | Davis | | Willows | | Petaluma | | Fernbridge | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 41 samples | | 36 samples | | 86 samples | | 38 samples | | 35 samples | | 22 samples | |
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| | *(g/100 g. milk)* | | | | | | | | | | | |
| 1. Total solids | 12.40 | 0.33 | 12.53 | 0.38 | 12.59 | 0.21 | 12.82 | 0.38 | 13.59 | 0.21 | 13.98 | 0.51 |
| 2. Fat | 3.82 | 0.22 | 3.90 | 0.27 | 3.85 | 0.17 | 4.11 | 0.32 | 4.64 | 0.26 | 4.96 | 0.45 |
| 3. S.N.F. | 8.58 | 0.15 | 8.63 | 0.24 | 8.74 | 0.10 | 8.71 | 0.16 | 8.95 | 0.22 | 9.02 | 0.21 |
| | *(mg./100 g. milk)* | | | | | | | | | | | |
| 4. Total nitrogen | 520.7 | 17.4 | 523.9 | 18.7 | 538.2 | 15.9 | 541.3 | 18.3 | 571.2 | 16.6 | 598.5 | 33.5 |
| 5. Casein N | 397.8 | 15.6 | 402.1 | 17.0 | 410.7 | 13.3 | 415.1 | 14.9 | 445.7 | 13.9 | 461.0 | 26.1 |
| 6. Lactalbumin + lactoglobulin N | 70.4 | 3.7 | 70.9 | 2.8 | 75.3 | 4.0 | 73.3 | 5.0 | 74.1 | 3.5 | 79.7 | 7.8 |
| 7. Proteose-peptone N | 21.2 | 3.0 | 20.0 | 3.1 | 20.1 | 2.1 | 21.7 | 3.0 | 22.5 | 3.9 | 26.0 | 4.8 |
| 8. Nonprotein N | 31.4 | 1.6 | 30.9 | 2.3 | 32.1 | 2.4 | 31.2 | 2.3 | 28.9 | 2.5 | 31.9 | 2.5 |
| 9. Total phosphorus | 93.4 | 3.6 | 94.0 | 3.9 | 94.9 | 3.8 | 96.1 | 4.2 | 102.9 | 4.7 | 103.8 | 6.0 |
| 10. Total soluble P | 42.7 | 3.3 | 42.5 | 3.1 | 43.4 | 2.9 | 43.0 | 4.2 | 44.3 | 3.5 | 44.5 | 4.4 |
| 11. Total inorganic P | 61.0 | 3.8 | 59.8 | 3.7 | 60.8 | 2.8 | 61.0 | 3.1 | 66.9 | 3.4 | 66.6 | 4.0 |
| 12. Soluble inorganic P | 32.9 | 2.3 | 32.3 | 1.6 | 32.6 | 1.8 | 31.8 | 2.7 | 33.8 | 2.5 | 34.6 | 3.4 |
| 13. Total organic P | 32.4 | 3.6 | 34.2 | 3.1 | 34.1 | 4.2 | 34.8 | 4.7 | 35.9 | 4.4 | 37.2 | 4.2 |
| 14. Soluble organic P | 9.9 | 2.9 | 10.2 | 2.6 | 10.9 | 3.4 | 11.2 | 2.9 | 10.5 | 3.3 | 10.0 | 2.2 |
| 15. Insoluble organic P | 22.5 | 3.6 | 24.0 | 3.1 | 23.2 | 4.6 | 23.9 | 4.5 | 25.5 | 5.6 | 27.2 | 3.7 |
| 16. Colloidal inorganic P | 28.1 | 4.0 | 27.6 | 3.7 | 28.2 | 3.3 | 29.2 | 2.6 | 33.1 | 3.2 | 32.0 | 3.9 |
| 17. Total insoluble P | 50.6 | 3.0 | 51.6 | 3.8 | 51.4 | 3.1 | 53.1 | 3.8 | 58.6 | 4.9 | 59.2 | 4.9 |
| 18. Citric acid | 148.0 | 13.3 | 148.8 | 14.0 | 148.1 | 14.1 | 150.6 | 15.8 | 153.6 | 15.1 | 150.5 | 21.4 |
| 19. Total Ca + Mg | 145.8 | 8.9 | 147.5 | 8.4 | 145.0 | 7.5 | 149.8 | 5.2 | 159.1 | 6.5 | 161.6 | 6.9 |
| 20. Total Ca | 130.7 | 3.5 | 131.9 | 3.5 | 131.2 | 4.0 | 136.0 | 4.0 | 145.4 | 3.0 | 149.3 | 4.8 |
| 21. Total Mg | 9.1 | 4.9 | 9.4 | 5.1 | 8.4 | 4.3 | 8.4 | 3.3 | 8.3 | 4.1 | 7.5 | 3.1 |
| 22. Soluble Ca | 49.4 | 2.4 | 49.0 | 3.0 | 48.0 | 3.6 | 48.7 | 2.6 | 51.4 | 4.2 | 53.3 | 5.8 |
| 23. Insoluble Ca | 81.3 | 3.9 | 83.0 | 4.9 | 83.2 | 4.2 | 87.3 | 4.7 | 93.9 | 4.2 | 95.9 | 5.1 |

TABLE 2

Significance [a] of differences in milk composition from different areas

| | Areas [b] ranked in order of increasing means | | | | | |
|---|---|---|---|---|---|---|
| 1. Total solids | V | N | D | W | P | F |
| 2. Fat | V | D | N | W | P | F |
| 3. S.N.F. | V | N | W | D | P | F |
| 4. Total nitrogen | V | N | D | W | P | F |
| 5. Casein N | V | N | D | W | P | F |
| 6. Lactalbumin + lactoglobulin N | V | N | W | P | D | F |
| 7. Proteose-peptone N | N | D | V | W | P | F |
| 8. Nonprotein N | P | N | W | V | F | D |
| 9. Total phosphorus | V | N | D | W | P | F |
| 10. Total soluble P | N | V | W | D | P | F |
| 11. Total inorganic P | N | D | V | W | F | P |
| 12. Soluble inorganic P | W | N | D | V | P | F |
| 13. Total organic P | V | D | N | W | P | F |
| 14. Soluble organic P | No significant differences | | | | | |
| 15. Insoluble organic P | V | D | W | N | P | F |
| 16. Colloidal inorganic P | N | V | D | W | F | P |
| 17. Total insoluble P | V | D | N | W | P | F |
| 18. Citric acid | No significant differences | | | | | |
| 19. Total Ca + Mg | D | V | N | W | P | F |
| 20. Total Ca | V | D | N | W | P | F |
| 21. Total Mg | No significant differences | | | | | |
| 22. Soluble Ca | D | W | N | V | P | F |
| 23. Insoluble Ca | V | N | D | W | P | F |

[a] Any two areas not underscored by the same line are significantly different.
Any two areas underscored by the same line are not significantly different.

[b] V—Visalia; N—Newman; D—Davis; W—Willows; P—Petaluma; and F—Fernbridge.

Any two areas not underscored by the same line are significantly different.
Any two areas underscored by the same line are not significantly different.
[b] V—Visalia; N—Newman; D—Davis; W—Willows; P—Petaluma; and F—Fernbridge.

TABLE 3
Composition of milk by season

| | I [a] | | II [b] | | III [c] | | IV [d] | |
|---|---|---|---|---|---|---|---|---|
| | 75 samples | | 58 samples | | 45 samples | | 80 samples | |
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| | *(g/100 g. milk)* | | | | | | | |
| 1. Total solids | 12.74 | 0.51 | 12.55 | 0.55 | 12.86 | 0.59 | 13.13 | 0.57 |
| 2. Fat | 4.02 | 0.36 | 3.85 | 0.38 | 4.13 | 0.49 | 4.31 | 0.47 |
| 3. S.N.F. | 8.72 | 0.19 | 8.70 | 0.27 | 8.73 | 0.19 | 8.81 | 0.19 |
| | *(mg./100 g. milk)* | | | | | | | |
| 4. Total nitrogen | 542.3 | 26.2 | 524.7 | 24.1 | 543.6 | 27.0 | 558.2 | 29.4 |
| 5. Casein N | 415.8 | 22.4 | 402.6 | 22.8 | 415.8 | 23.1 | 429.6 | 24.2 |
| 6. Lactalbumin + lactoglobulin N | 73.3 | 5.6 | 70.9 | 3.2 | 75.3 | 4.4 | 75.6 | 5.0 |
| 7. Proteose-peptone N | 21.7 | 3.6 | 20.4 | 2.8 | 21.6 | 2.9 | 21.5 | 4.1 |
| 8. Nonprotein N | 31.4 | 2.2 | 30.8 | 2.2 | 31.0 | 2.1 | 31.5 | 3.0 |
| 9. Total phosphorus | 98.5 | 5.6 | 95.1 | 6.5 | 94.8 | 4.7 | 96.7 | 4.6 |
| 10. Total soluble P | 45.5 | 2.9 | 43.5 | 3.1 | 41.5 | 3.4 | 42.3 | 3.2 |
| 11. Total inorganic P | 63.4 | 4.1 | 61.7 | 4.5 | 60.8 | 3.3 | 61.8 | 4.1 |
| 12. Soluble inorganic P | 33.6 | 2.5 | 33.8 | 2.3 | 32.3 | 1.8 | 31.6 | 2.1 |
| 13. Total organic P | 35.1 | 4.0 | 33.4 | 4.5 | 34.1 | 3.7 | 34.8 | 4.4 |
| 14. Soluble organic P | 11.8 | 2.9 | 9.7 | 3.0 | 9.2 | 3.1 | 10.6 | 2.7 |
| 15. Insoluble organic P | 23.3 | 4.2 | 23.8 | 4.7 | 24.8 | 4.3 | 24.3 | 4.7 |
| 16. Colloidal inorganic P | 29.8 | 3.8 | 27.8 | 3.8 | 28.5 | 2.9 | 30.2 | 4.2 |
| 17. Total insoluble P | 53.0 | 4.2 | 51.6 | 5.4 | 53.3 | 4.7 | 54.5 | 4.7 |
| 18. Citric acid | 153.9 | 14.2 | 156.6 | 11.3 | 145.7 | 12.1 | 142.4 | 16.5 |
| 19. Total Ca + Mg | 150.1 | 10.0 | 147.4 | 8.5 | 148.4 | 9.4 | 151.1 | 9.3 |
| 20. Total Ca | 133.8 | 7.0 | 134.1 | 7.1 | 136.8 | 7.0 | 137.1 | 7.8 |
| 21. Total Mg | 9.8 | 5.2 | 8.1 | 3.4 | 7.1 | 4.6 | 8.5 | 3.3 |
| 22. Soluble Ca | 49.0 | 3.6 | 50.1 | 4.0 | 49.7 | 3.7 | 49.0 | 4.2 |
| 23. Insoluble Ca | 84.8 | 6.2 | 84.0 | 7.2 | 87.1 | 5.3 | 88.0 | 6.7 |

[a] February, March, and April; [b] May, June, and July; [c] August, September, and October; [d] November, December, and January.

TABLE 4

Significance[a] of seasonal differences in milk composition

| | Seasons ranked in order of increasing means | | | |
|---|---|---|---|---|
| 1. Total solids | II[b] | I[c] | III[d] | IV[e] |
| 2. Fat | II | I | III | IV |
| 3. S.N.F. | II | I | III | IV |
| 4. Total nitrogen | II | I | III | IV |
| 5. Casein N | II | III | I | IV |
| 6. Lactalbumin + lactoglobulin N | II | I | III | IV |
| 7. Proteose-peptone N | No significant differences | | | |
| 8. Nonprotein N | No significant differences | | | |
| 9. Total phosphorus | III | II | IV | I |
| 10. Total soluble P | III | IV | II | I |
| 11. Total inorganic P | III | II | IV | I |
| 12. Soluble inorganic P | IV | III | I | II |
| 13. Total organic P | No significant differences | | | |
| 14. Soluble organic P | III | II | IV | I |
| 15. Insoluble organic P | No significant differences | | | |
| 16. Colloidal inorganic P | II | III | I | IV |
| 17. Total insoluble P | II | I | III | IV |
| 18. Citric acid | IV | III | I | II |
| 19. Total Ca + Mg | II | III | I | IV |
| 20. Total Ca | I | II | III | IV |
| 21. Total Mg | III | II | IV | I |
| 22. Soluble Ca | No significant differences | | | |
| 23. Insoluble Ca | II | I | III | IV |

[a] Any two seasons not underscored by the same line are significantly different. Any two seasons underscored by the same line are not significantly different; [b] May, June, and July; [c] February, March, and April; [d] August, September, and October; [e] November, December, and January.

TABLE 5
F values obtained by analysis of variance

| | Season | Area |
|---|---|---|
| 1. Total solids | 71.66** | 231.65** |
| 2. Fat | 62.41** | 178.88** |
| 3. S.N.F. | 6.67** | 34.45** |
| 4. Total nitrogen | 56.26** | 117.01** |
| 5. Casein N | 50.89** | 119.78** |
| 6. Lactalbumin + lactoglobulin N | 17.84** | 21.23** |
| 7. Proteose-peptone N | 2.17 NSD [a] | 15.12** |
| 8. Nonprotein N | 1.37 NSD | 9.84** |
| 9. Total phosphorus | 11.17** | 42.57** |
| 10. Total soluble P | 20.09** | 2.28* |
| 11. Total inorganic P | 6.95** | 32.16** |
| 12. Soluble inorganic P | 17.81** | 6.94** |
| 13. Total organic P | 2.18 NSD | 5.20** |
| 14. Soluble organic P | 9.72** | 1.28 NSD |
| 15. Insoluble organic P | 1.41 NSD | 4.60** |
| 16. Colloidal inorganic P | 6.97** | 16.60** |
| 17. Total insoluble P | 6.92** | 35.81** |
| 18. Citric acid | 15.09** | .92 NSD |
| 19. Total Ca + Mg | 3.18* | 32.69** |
| 20. Total Ca | 15.80** | 168.39** |
| 21. Total Mg | 4.53** | .81 NSD |
| 22. Soluble Ca | 1.47 NSD | 10.60** |
| 23. Insoluble Ca | 13.60** | 72.54** |

** Season F.01 = 3.88. Area F.01 = 3.11.
* Season F.05 = 2.65. Area F.05 = 2.26.
[a] NSD — no significant difference.

The F values resulting from analysis of variance of the data are given in Table 5. They indicate the tremendous significance of the compositional differences among these commercial bulk milk samples, both seasonally and within areas. Usually, the variation in composition due to origin of the milk (area, breed, feed, etc.) is greater than the variation due to changes associated with season (temperature feed, stage of lactation, etc.). The exceptions are total soluble phosphorus, soluble inorganic phosphorus, soluble organic phosphorus, citric acid, and magnesium. With these five constituents, seasonal changes have a greater significance than area changes. This would imply that these constituents might well be implicated with the seasonal variations in milk that result in processing difficulties or defective dairy products.

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# COMPARATIVE ACCURACY OF THE PLASTIC BEAD METHOD OF GOLDING AND THE LACTOMETER METHOD OF WATSON FOR ROUTINE DETERMINATION OF SOLIDS-NOT-FAT IN MILK[1]

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**SUMMARY**

The plastic bead method of Golding was evaluated as a rapid test for solids-not-fat in the milk of individual cows. Ten plastic beads each varying 1 mg. in density in the range of 1.025 to 1.034 are placed in a 4-oz. sample jar fitted with a plastic grid and a metal screw-cap. Milk is sampled directly into the sample jar and after the fat has been liquefied by heating to 104° F. and holding for 5 min., the test is made by counting the number of sinking beads at 68° F. A formula for converting the number of sinking beads to per cent SNF was calculated, using 1,873 milk samples from Guernsey, Holstein, and Jersey cows of 53 herds. This formula is: per cent SNF $= 9.13 - 0.279\ B + 0.307\%\ F$ where $B$ is the number of plastic beads sinking at 68° F. and per cent $F$ is the percentage fat in the milk. In a second experiment, plastic beads and the lactometer of Watson were compared, using gravimetric assays for reference. On 300 milk samples from six breeds the standard deviation from regression was 0.17% SNF for the lactometer and 0.18% SNF for the plastic beads. The plastic bead method is sufficiently accurate for the routine testing of milk of individual cows for per cent SNF. The method is simple and rapid and requires no more milk than is routinely sampled for DHIA testing.

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A rapid and practical solids-not-fat (SNF) test for cow's milk has four major uses. These are: (a) for purposes of selective breeding, since SNF level in cow's milk is heritable (11, 15, 18, 20, 22); (b) pricing of milk on its total composition; (c) rapid determination of solids in certain manufacturing processes; and (d) screening samples of milk for possible water adulteration, so that the suspected ones may be further tested by the cryoscope (14).

Hydrometric techniques have been extensively investigated over a period of years. Many types of instruments, conditions of testing, and sources of milk have been used. As a result, many different formulae for conversion of density readings to SNF have been developed. Several excellent comprehensive reviews of techniques and formulae available are referred to by Watson (22).

The lactometer method used for 50 yr. has become the most common method for determining the density of milk (13) and its errors and inaccuracies have been carefully tabulated (16). The use of these instruments of various types has not been extensive, as might be expected, because of fragility, sensitivity to temperature changes, time required for testing large numbers of samples, and the quantity of milk required. Recently, Watson developed a lactometer which has been tested for accuracy for determining total solids (12, 22). This particular instrument requires about 8 oz. of milk for a density determination. A smaller instrument requiring about 4 oz. of milk has been developed and is

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considered sufficiently accurate for the testing of individual cows' milk for purposes of selective breeding (11).

In 1957, Golding (6) first reported the development of an ingenious method for rapidly determining SNF with plastic density beads. These have subsequently been referred to as density beads (7), plastic hydrometers (8), and subsurface hydrometers (4). Polymethylstyrene (CYMAC 400) was selected after extensively testing many plastics (6). Certain qualities of CYMAC 400 made it especially suitable. It is constant in density when annealed, practically the same as water in coefficient of thermal expansion between 66 and 76° F., easy to mold into the required shape by pressure injection, and completely insoluble in water, milk, and detergents (6). It has a density of 1.023 which changes only slightly on annealing (6). The plastic is brought to the desired density by mixing with fine barium sulfate and the desired insoluble color (6). Each colored bead, 5/16 in. in diameter, varies in density by 1 mg. per milliliter at 68° F. The lowest density bead has a specific gravity of 1.025 and the highest 1.034, making a total of ten beads, each of a different color (6). A set of these is placed in a 4-oz. sample jar fitted with a snap-in plastic grid and a white lacquered screw-cap (6-8). Each bead represents a density of one lactometer reading between temperatures of 68 to 76° F.

Preliminary field testing of the density plastic beads involving bulk herd milks and individual cows in the University herd indicated that this simple instrument offered exceptional promise as a routine procedure for testing SNF (8). It also became evident from early field trials that the British Standards formula (3), as used in preliminary studies (7, 8), was not entirely suitable for converting plastic bead assays to SNF when milk from individual cows was used. The purpose of this experiment was twofold, namely: (a) To develop an average conversion formula based on milk samples from many cows in different herds under variable environments; (b) to compare the relative accuracy of the plastic beads and the lactometer, using the gravimetric assay for reference values.

## EXPERIMENTAL METHODS

*Source of milk.* Milk samples were taken during July and August, 1959, by DHIA supervisors, using 4-oz. sample jars containing the plastic beads (7). The 53 cooperator herds involved in Experiment I represented Guernsey, Holstein, and Jersey breeds. In Experiment II, 1 pt. of milk from 45 to 52 cows from each of six breeds was sampled by our laboratory technicians. Approximately 750 different Golding plastic bead hydrometers (7, 8) were used during the experiments.

*Measurements.* All measurements were made at the same time in our laboratories and each was a single assay. The Babcock method was used for determining fat percentage and recorded to the nearest 0.1%. Total solids, measured gravimetrically (2), served as reference values for SNF by subtracting the percentage of fat. The number of Golding plastic beads sinking at 68° F. was recorded for all samples. Protein was determined on about two-thirds of the samples from Experiment I, using dye binding (1, 19). In addition to the tests

listed for Experiment I, Watson lactometer (22) determinations were made on samples in Experiment II.

*Experiment I.* The milk was brought to 104° F. and held for 5 min. During this time thorough mixing was done. The temperature of the milk was lowered to 68° F. and the number of plastic beads sinking was recorded. Occasionally, one bead near the exact density of the milk remained in the middle, as determined by checking the number floating. When this happened a slight rotary mixing often resulted in the bead either rising or sinking. In any case the number actually sinking was recorded. Immediately following, the milk was sampled for fat, gravimetric total solids (2), and protein assay (1, 19).

*Experiment II.* The procedure was similar to Experiment I, except that the milk was tempered in 1-pt. bottles and the lactometer reading taken at 102° F. before transferring a portion to the plastic bead sample jars.

Statistical procedures as outlined by Snedecor (17) in Chapters 6 and 13 were used.

## RESULTS AND DISCUSSION

*Experiment I.* Multiple regression formulae for estimating SNF from the number of plastic beads sinking was determined for the Guernsey, Holstein, and Jersey breeds (Table 1).

TABLE 1

Multiple regression formulae for estimating SNF from plastic beads

| | | | Estimated SNF = $a - bB + b\ \%\ F$[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $a$ | | $bB$ | | $b\ \%\ F$[a] | |
| Breed | Herds | Samples | 95% int.[b] | | 95% int.[b] | | 95% int.[b] | |
| | | *(No.)* | *(%)* | | | | | |
| Guernsey | 18 | 457 | 9.17 | 0.002 | 0.274 | 0.013 | 0.293 | 0.022 |
| Holstein | 21 | 917 | 9.12 | 0.002 | 0.279 | 0.006 | 0.313 | 0.011 |
| Jersey | 14 | 499 | 9.27 | 0.004 | 0.277 | 0.008 | 0.286 | 0.011 |
| Total | 53 | 1,873 | 9.13 | 0.002 | 0.279 | 0.006 | 0.307 | 0.008 |

[a] $a$ is the constant; $b$ is the partial regression; $B$ is the number of beads sinking; per cent $F$ is the percentage fat in the milk.

[b] (Standard error) ($t.05n$).

The partial regressions for gravimetric SNF on numbers of plastic beads sinking independent of percentage fat are essentially constant for the three breeds. The partial regressions for gravimetric SNF on fat percentage independent of plastic beads sinking show more variability. The Jersey regression is lower than the Holstein's or total (Table 1). However, with either one or ten beads sinking, the fat percentage would have to be as low as 2% or as high as 10% before the conversion error would exceed 0.11% when formulae for each breed are compared with the formula for total samples. Extremes of this kind would occur only rarely and then generally not consistently for the same cow. It is concluded that one formula is adequate for these three breeds.

The plastic bead values were converted to estimated SNF. The averages for this study, as shown in Table 2, indicate no serious bias for breeds. Plastic bead SNF differed from gravimetric SNF by more than 0.2% in 25% of the 1,873

TABLE 2
Percentages of SNF, fat, and protein in the milk used for Experiment I

| Breed | % SNF | | | | % Fat | | % Protein | | |
|---|---|---|---|---|---|---|---|---|---|
| | Gravimetric | | Plastic beads | | | | | | |
| | Samples | $x$ | $s$* | $x$ | $s$* | $F$ | $s$* | Samples | $x$ | $s$* |
| | (No.) | (%) | | | | | | (No.) | (%) | |
| Guernsey | 457 | 8.99 | 0.44 | 8.99 | 0.41 | 4.68 | 0.79 | 328 | 3.40 | 0.38 |
| Holstein | 917 | 8.50 | 0.46 | 8.50 | 0.42 | 3.62 | 0.50 | 658 | 3.05 | 0.26 |
| Jersey | 499 | 9.36 | 0.42 | 9.33 | 0.38 | 5.33 | 0.87 | 402 | 3.82 | 0.34 |
| Total | 1,873 | 8.84 | 0.58 | 8.84 | 0.54 | 4.33 | 1.00 | 1,388 | 3.36 | 0.46 |

* Standard deviation.

samples. There was little difference between the three breeds (Guernseys 24%, Jerseys 26%, and Holsteins 25%). Jerseys were estimated low more frequently than high at a ratio of 3.2.

Waite (21) has found that casein is the factor primarily influencing the accuracy of SNF determined by density measurements, a high ratio of casein to lactose leading to low per cent SNF. Since lactose is less variable between breeds than protein (9), one would predict that on an average the three-breed conversion formula would tend to estimate SNF too low for those samples high in protein. This is likely the reason why a few more Jersey samples were estimated low in SNF as compared with the number estimated too high. The importance of considering per cent protein in the milk sample was evaluated from a four-factor multiple regression analysis. The partial regression coefficient for gravimetric SNF on per cent protein, independent of number of beads sinking and per cent fat, was + 0.107. Thus, a variation of 1% in protein above or below the mean would, on an average, offset the estimated per cent SNF 0.107%.

The average protein for cows in this study was 3.36%. For a lactation period few cows average below 2.8% or higher than 4.2%. Percentage protein may exceed five at the first test period in early lactation and lactose is lowest when protein is highest (9). On a lactation basis it is unlikely that a conversion error of more than ± 0.1% SNF would occur. For application to mass testing of cows on DHIA the consideration of per cent protein in a SNF conversion formula is

TABLE 3
The average SNF and standard deviations ($s$) as estimated by the gravimetric, lactometer, and plastic bead methods

| Breed | Samples | Gravimetric | | Lactometer | | Plastic beads | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | $s$ | $\bar{x}$ | $s$ | $\bar{x}$ | $s$ |
| | (No.) | (% SNF) | | | | | |
| Ayrshire | 50 | 8.77 | 0.47 | 8.74 | 0.44 | 8.77 | 0.49 |
| Brown Swiss | 52 | 9.01 | 0.49 | 9.04 | 0.38 | 9.07 | 0.39 |
| Guernsey | 51 | 8.86 | 0.37 | 8.98 | 0.33 | 8.96 | 0.36 |
| Holstein | 52 | 8.56 | 0.41 | 8.60 | 0.38 | 8.55 | 0.36 |
| Jersey | 50 | 9.52 | 0.41 | 9.64 | 0.40 | 9.69 | 0.43 |
| Milking Shorthorns | 45 | 8.57 | 0.52 | 8.51 | 0.47 | 8.56 | 0.51 |
| Total | 300 | 8.89 | 0.55 | 8.92 | 0.55 | 8.94 | 0.57 |

TABLE 4

Covariance comparisons between gravimetric (G), lactometer (L), and plastic bead (B) SNF estimates

| Breed | Samples | Comparisons[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | L × G | | | | B × G | | | | B × L | | | |
| | | r | *bG. L* | *s b* | *sG. L* | r | *bG. B* | *s b* | *sG. B* | r | *bL. B* | *s b* | *sL. B* |
| | *(No.)* | | (%) | | | | (%) | | | | (%) | | |
| Ayrshire | 50 | 0.96 | 1.04 | 0.045 | 0.14 | 0.94 | 0.92 | 0.047 | 0.16 | 0.95 | 0.86 | 0.039 | 0.14 |
| Brown Swiss | 52 | 0.93 | 1.21 | 0.069 | 0.19 | 0.93 | 1.16 | 0.063 | 0.18 | 0.98 | 0.94 | 0.026 | 0.07 |
| Guernsey | 51 | 0.92 | 1.02 | 0.064 | 0.15 | 0.91 | 0.94 | 0.062 | 0.16 | 0.96 | 0.89 | 0.037 | 0.10 |
| Holstein | 52 | 0.92 | 0.98 | 0.059 | 0.16 | 0.88 | 0.98 | 0.074 | 0.19 | 0.93 | 0.97 | 0.053 | 0.14 |
| Jersey | 50 | 0.95 | 0.98 | 0.046 | 0.13 | 0.93 | 0.90 | 0.051 | 0.15 | 0.97 | 0.91 | 0.035 | 0.10 |
| Milking Shorthorns | 45 | 0.95 | 1.03 | 0.055 | 0.17 | 0.94 | 0.95 | 0.055 | 0.19 | 0.98 | 0.90 | 0.030 | 0.10 |
| Common | 294[b] | 0.94 | 1.04 | 0.073 | 0.16 | 0.92 | 0.97 | 0.075 | 0.18 | 0.96 | 0.91 | 0.015 | 0.11 |
| Total | 298[c] | 0.95 | 0.96 | 0.057 | 0.17 | 0.95 | 0.91 | 0.057 | 0.18 | 0.98 | 0.93 | 0.012 | 0.12 |

[a] Where r is the correlation coefficient, *b* is the regression, *s b* is the standard deviation of the regression coefficient, and *sG. L*, *G. B*, and *L. B* is the sample standard deviation from regression.

[b] N–6.

[c] N–2.

impractical unless such conversion is done with electronic computers. In this case the suggested formula is:

$$\text{SNF} = 9.13 - 0.279\,B + 0.30\%\,F + [0.107\ (\%\ \text{P} - 3.36)]$$

where $B$ is the number of sinking plastic beads, per cent $F$ is per cent fat, per cent $P$ is per cent protein, and 3.36 is the average per cent protein (Table 2). Through programming for electronic computation, per cent protein can be considered when known.

*Experiment II.* The plastic bead formula developed in Experiment I was used to compare its accuracy against the lactometer (22) and gravimetric assays for SNF. Table 3 shows that the average per cent SNF by each of the methods was similar for each of the breeds, except for Guernseys. For this breed, average SNF for gravimetric assay was 8.86%, with 95% confidence limits from 8.75 to 8.97%. The upper limit exceeds the 8.96% SNF average for plastic beads, but misses the 8.98% SNF average for lactometer estimates.

Data from a covariance analysis show no essential difference in accuracy between lactometer and plastic bead SNF as compared with the gravimetric assay. The standard deviation from regression for the 300 samples (d.f. = 298) was 0.17% SNF for the lactometer (22) and 0.18% SNF for the plastic beads. The difference in slope of the regression coefficients, as determined from the ratios between the variance for regression coefficients and the within breeds variance was different at the 10% level of probability for lactometer vs. gravimetric SNF and at the 5% level for plastic beads vs. gravimetric SNF. The regressions for Brown Swiss (Table 4) are different from the rest ($P < 0.05$) for both the lactometer and plastic bead methods compared with the gravimetric assay. For this breed the lactometer and plastic bead SNF varied from gravimetric in a similar direction.

The results of Experiment II show there is no difference in relative accuracy between the lactometer (22) and plastic bead SNF when comparisons are made against single gravimetric assays.

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# AN IMPROVED PROCEDURE FOR THE DETERMINATION OF MILK PROTEINS BY DYE BINDING [1]

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**SUMMARY**

The precision of the Orange G dye binding method has been increased by a new method for measuring the concentration of dye not bound by the milk proteins. The results of 345 comparisons of protein determined by the new method with that determined by the macro-Kjeldahl showed a correlation coefficient of .98, with a standard deviation from regression of 0.078% protein. When the protein contents of the milk from individual cows of six different breeds were determined by the two methods, covariance analysis showed no significant breed difference. From 224 duplicate determinations it was observed that 19 out of every 20 single determinations fell within 0.06% protein of the average for duplicate readings.

Large variations in the amounts of butterfat and lactose were found to be without effect on the protein values determined by dye binding. The only preservative found suitable for use in raw milk at room temperature was mercuric chloride. The mixture of 1.5 ml. of milk with 25 ml. of dye solution was stable for 2 wk. at room temperature.

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The need for a rapid routine method for the determination of milk proteins has become increasingly evident as more emphasis is placed on the importance of the nonfat milk solids. Udy (7) first applied the dye binding procedure to the determination of flour proteins. Later, he applied the same procedure to the determination of milk proteins with our cooperation (8). The dye used was Orange G, a disulphonic acid type, which binds the basic groups of proteins maintained at a pH of about 2.

Schober and Hetzel (3) have described a similar method using another dye, amino black 10B. Both dyes form insoluble dye-protein complexes. The precipitate formed by the dye and the milk protein can be removed by filtering or centrifuging. The optical density of the supernatant liquid is then read in a spectrophotometer. The German workers investigated several factors affecting the test. They found that above pH 3.5 the precipitation of the milk protein was not quantitative. The optical density of the supernatant increased slightly with temperature in the range of 0–40° C. Lower optical densities were secured when the pH of the citrate-phosphate was decreased from 2.8 to 1.9. This means that more dye was bound by the protein at the lower pH values. The dye was precipitated from filtrates of milk treated with trichloracetic acid, indicating that some nonprotein nitrogen was bound. When free amino acids were added, however, only arginine changed the optical density when added at levels as high as 1%. The addition of peptones and higher peptides caused a significant decrease in the optical density. A doubling of the amount of lactose in milk did not affect the amount of dye

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bound, but the addition of 1% calcium chloride caused a 25% decrease in the optical density of the supernatant.

Steinholtz (5) confirmed the practicality of the Schober and Hetzel procedure (3). From 64 samples preserved with mercuric chloride and 62 unpreserved herd milk samples, the correlation between Kjeldahl protein and optical density of the supernatant dye solution was $-0.982$ for the preserved and $-0.975$ for the unpreserved samples. The calculated confidence interval at the 95% level was 0.12% protein within the range of 2.8 to 4% total protein. Comparatively, the correlation for formol titration was 0.895 and 0.614 when protein was calculated from fat content.

Recently, Treece *et al.* (6) found a correlation coefficient of 0.98 between the method of Udy (8) and the Kjeldahl method. They also state that seldom did a sample vary as much as $+0.1$ percentage unit from the Kjeldahl value and aproximately two-thirds of the samples fell within $\pm 0.05$ percentage units of the corresponding Kjeldahl value.

In a preliminary report (1), it was shown that the method of Udy (8) was practical, although the amount of dye bound per unit weight by the different milk proteins varied greatly. The present paper describes an improved procedure which has given the dye binding method the same precision as the macro-Kjeldahl method.

## EXPERIMENTAL PROCEDURE

*Apparatus and reagent.*

(a) Beckman Model B Spectrophotometer with special test tube holder compartment, or similar instrument.

(b) Flow-through cuvette to fit the test tube holder. The one developed by us (1) consists of a U-tube arrangement (Figure 1), so that the sample flows downward through the absorbance section, then upward and out of the instrument. The constricted part of the U-tube has a light path of approximately 0.75 mm. The design is such that the capacity of the U-tube is about 5 ml., which allows for rapid rinsing out of one sample by the next. Tubing must be large enough to prevent accumulation of air bubbles in the system. Each cuvette should be standardized as described under "Standard Curve."

(c) Hypodermic syringe, 2-ml. size.[3]

(d) Automatic saline dispenser[3] which allows adjustment of the size of the sample delivered to $1.500 \pm .003$ ml.

(e) Automatic pipette, size 25.00 ml., for delivery of the dye reagent.

(f) 50-ml. Erlenmeyer flasks or similar containers for mixing dye and milk samples. These can also be used for collecting the filtrate and are convenient for pouring it through the cuvette.

(g) Medium density filter paper. Size 11 cm. S & S No. 597 paper is satisfactory.

(h) Reagent. The certified Orange G dye (C. I. No. 27) should assay at least 95%. The reagent is prepared by dissolving exactly 1.000 g. of the dye, corrected

[3] Becton Dickinson & Co., Rutherford, N.J.

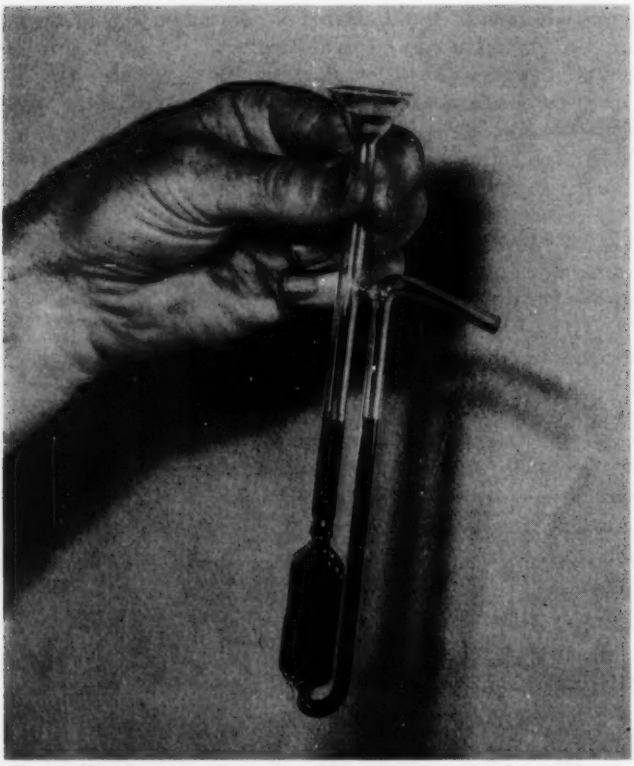

FIG. 1. The flow-through cuvette. Light path constricted to 0.75 mm.

for its assay, in a solution containing 21.0 g. of citric acid and 2.5 ml. of a 10% thymol solution in alcohol (used as a preservative). The final volume is made up to 1,000 ml. When dye binding capacities are to be measured, it is desirable to recrystallize the dye from hot 90% ethanol, then dry to constant weight. A standard curve relating dye concentration to spectrophotometric absorbance can then be plotted. Maximum absorbance values are secured at a wave length of 0.475 $\mu$.

*Method.*

For milk the syringe is first calibrated to deliver 1.500 $\pm$ .003 ml. by weighing several samples of distilled water delivered from the syringe. The milk samples are then measured into 50-ml. containers and 25 ml. of the dye reagent added. The mixture is stoppered and shaken for about 15 sec. and allowed to stand for 30 min. before filtering through a 11-cm. filter paper.

The spectrophotometer is adjusted to a wave length of 0.475 $\mu$. A standard dye solution containing 0.500 mg. per milliliter is prepared by diluting 50 ml. of the reagent dye solution to 100 ml. This solution is used to standardize

the instrument. After rinsing out the flow-through cuvette with about 25 ml. of the 1:2 dye solution, the slit width is opened until the absorbance decreases to 0.500 with the sensitivity set at one (or zero at sensitivity of 2). This method of standardization allows for maximum precision in the useful range of dye concentration which is 0.4–0.6 mg. per milliliter. It is based upon the principle of relative absorbance described by Hiskey *et al.* (2). The filtrates prepared from the milk and dye reaction can then be poured through the cuvette and the absorbance values recorded. The per cent protein is read from a standard curve prepared for milk (see below).

Pouring at least 20 ml. of the filtrate through the cuvette rinses out the previous sample, giving a constant reading for the new one. The dye reagent is stable indefinitely. The milk and dye mixture can be stored 2 wk. at room temperature with no change in the dye binding results.

*Standard curve.*

A series of fresh milk samples provides a direct means for standardizing the dye binding procedure. Each sample is run by the macro-Kjeldahl method and the per cent total nitrogen multiplied by 6.38, which is commonly used to convert nitrogen to protein. The same samples are then run by the dye binding procedure. After the protein dye complex is removed by filtration the light absorbance of the filtrate is measured and plotted against the per cent protein found by the Kjeldahl method. Linear regression equations such as those shown in Table 1

TABLE 1

Relationship between Kjeldahl protein and absorbance readings (dye binding) for milk from different breeds

| Breed | N | Per cent protein Kjeldahl | Dye binding formulae [a] | | | Correlation [c] |
|---|---|---|---|---|---|---|
| | | | $a$ | $bX$ | $sb$ [b] | |
| Ayrshire | 50 | 3.43 | 5.68 | − 3.17X | 0.069 | 0.99 |
| M. Shorthorn | 45 | 3.23 | 5.85 | − 3.33X | 0.064 | 0.99 |
| Brown Swiss | 52 | 3.56 | 5.76 | − 3.23X | 0.062 | 0.99 |
| Jersey | 72 | 3.98 | 5.56 | − 3.02X | 0.123 | 0.92 |
| Guernsey | 76 | 3.53 | 5.57 | − 3.01X | 0.087 | 0.97 |
| Holstein | 50 | 3.25 | 5.75 | − 3.24X | 0.120 | 0.97 |
| Total | 345 | 3.53 | 5.60 | − 3.05X | 0.031 | 0.98 |
| Common | 339 [d] | ..... | 5.68 | − 3.16X | 0.037 | 0.97 |

[a] $a$ is the constant, $b$ is the regression coefficient, and $X$ is the absorbance reading at 0.475 $\mu$.
[b] Standard deviation of the regression coefficient.
[c] Kjeldahl protein compared with dye bound protein, calculated using the common formula.
[d] Degrees of freedom or N-6.

are then calculated. This method gives a direct comparison of the two methods, and the standard error of estimate is a measure of the precision of the dye binding procedure, assuming that the Kjeldahl protein values are the correct ones. Results to be reported were compared in this manner.

A convenient method for constructing the standard curve consists of carefully weighing samples of a standard milk powder, adding 1.5 ml. of water to each sample, and completing the dye binding procedure. From the known protein content of the powder a protein percentage can be calculated for each sample.

These values are then used to construct the curve. Since it has been established that the curve is linear within the useful range, two points only are required, one equivalent to about 30 mg. of protein and the other equivalent to about 60 mg. of protein.

*Dye binding capacity.*

Another means of standardizing the procedure lies in the calculation of the dye binding capacity (DBC) of the milk proteins. The DBC is defined as the milligrams of dye bound per milligram of protein (N × 6.38). This constant assumes a stoichiometric reaction between dye and protein, which is true within certain limits to be discussed.

The cuvette is first standardized by reading the absorbance of several known dilutions of a standard dye solution and constructing a curve such as A or B in Figure 2. The instrument has been standardized by adjusting the absorbance to read 0.500 with a dye dilution containing exactly 0.500 mg. of pure dye per milliliter. Curve A assumes a linear response of absorbance to dye concentration, which is true within the range of 0.4 to 0.6 mg. of dye per milliliter. For precise work over an extended range one should plot dye concentration against measured absorbance at frequent points. Curve B was plotted from such an experiment. Curve A and B diverge increasingly at points above a dye concentration of 0.6 mg/ml.

The lower limit of dye concentration which can be read from either Curve A or B is about 0.33 mg. per milliliter. This fact insures that an excess of dye is always present. Udy (8) has stated that the concentration of unbound dye should not "drop appreciably below 0.5 g. per L." Those who wish to measure dye concentrations below 0.33 mg. per milliliter can do so by setting the instrument at an absorbance of 1.00 with a dye solution containing 0.500 mg/ml. (Figure 2), Curve C. The curve marked D was constructed by first setting the instrument at zero absorbance with water.

The concentration of dye not bound by the protein is read from the dye standard curve. This value multiplied by the total volume (sample + dye volume) gives the total amount of dye left unbound in milligrams. The bound dye is found by subtracting this amount from the total amount of dye added (25.00 mg.). The difference is the amount of dye bound by the protein in the sample. The DBC is then calculated as the ratio of dye bound over protein (N × 6.38). When this ratio is known, its reciprocal can be used as a factor for multiplying the amount of dye bound by an unknown sample to find the amount of protein present. The results to be discussed show that when the protein content of the sample is above 60 mg. the DBC decreases with increasing protein.

## RESULTS AND DISCUSSION

*Fresh milk samples.*

To test the reproducibility and precision of the method, a large number of samples from individual cows were collected and analyzed for protein by both the macro-Kjeldahl and dye binding methods, using single determinations. Six

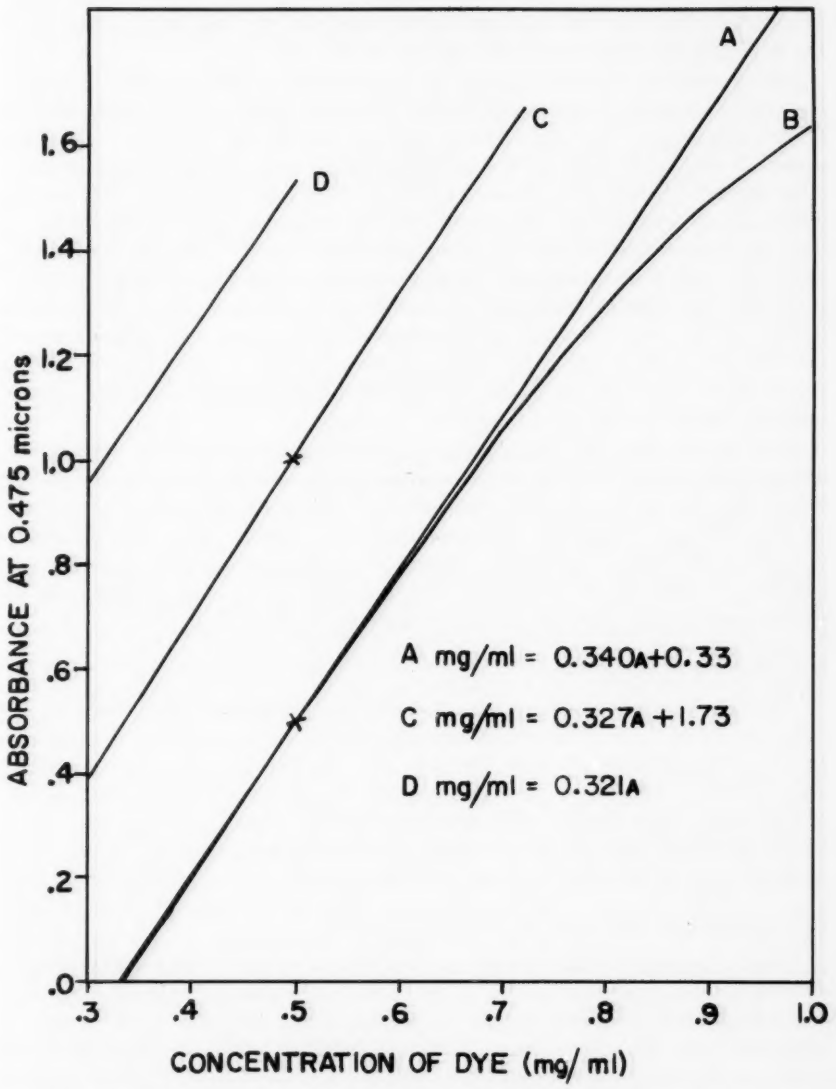


FIG. 2. Standard curves for measuring the concentration of unbound dye. Curve A—spectrophotometer set at an absorbance of 0.500 with dye concentration of 0.500 mg. per milliliter and assuming linear relation between absorbance and dye concentration. Curve B—same as A, except that it shows nonlinearity in the higher range of dye concentration. Curve C—spectrophotometer set at an absorbance of 1.00 with dye concentration of 0.500 mg/ml. Curve D—spectrophotometer set at zero with zero dye concentration.

different breeds of dairy cows were compared. Linear regression of the protein values determined by the Kjeldahl method against the absorbance readings of the unbound dye found in the filtrate was made.

The results are shown in Table 1, together with their statistical constants. By covariance analysis the breeds did not differ significantly in slope or elevation of the regressions. For the common regression equation (Table 1), per cent protein = 5.68 minus 3.16 $X$, the sample standard error of the regression coefficient is 0.037. The sample standard deviation from regression is 0.078% protein. The standard error of per cent protein estimated near the mean is 0.004% and is four times greater when protein in the milk exceeds 4.5%. The correlations between Kjeldahl per cent protein and per cent protein from dye binding, as calculated from the common formula, are also shown in Table 1. The correlation of 0.98 for all samples is identical to the results of Treece *et al.* (6). Jerseys showed the lowest correlation ($r = 0.92$) of the six breeds. Since Jerseys had the highest average per cent protein, this coincides with the greater error observed for samples high in protein.

Most likely, these variations are reflections of differences in the DBC of the different proteins in the milk of individual cows. A previous publication (1) has shown that the different protein fractions of milk vary in their capacity to bind the dye. Whey proteins bind more dye per unit weight than casein. The dialyzable nitrogen compounds (nonprotein nitrogen) bind none of the dye. The fact that the nitrogen distribution in normal milk is relatively constant accounts for the similar regression coefficients shown in Table 1.

*Repeatability of the method.*

During the study, duplicate dye binding determinations were made on 224 samples of milk from Ayrshires, Guernseys, Holsteins, and Jerseys. The correlations between duplicates varied from a low of 0.992 for Jerseys to a high of 0.997 for Holsteins. The accuracy of a single dye binding determination in 95% of the samples was within 0.67% protein of what the duplicate readings would have given. From an applied standpoint there appears no necessity for duplicate readings, once a technician has mastered the method.

*Dye binding capacity and total protein.*

Emphasis should be placed upon the fact that the relation between the protein content of the milk and the amount of dye bound is empirical. Figure 3 shows that less dye is bound per unit weight of protein when the amount of protein is increased above the normal range of values found in 1.5 ml. of milk. Curve A shown in Figure 3 was secured by weighing amounts of a nonfat milk powder (150–420 mg.) to give the calculated amounts of protein shown along the abscissa. These samples of powder were shaken with 25.00 ml. of the dye solution. The absorbance of the filtrate was determined after standardizing the instrument so that a 0.500 mg/ml standard dye solution read 1.000 instead of 0.500. This procedure allowed lower concentrations of dye to be measured (see Figure 2, Curve C). When the instrument was standardized in the normal manner, the

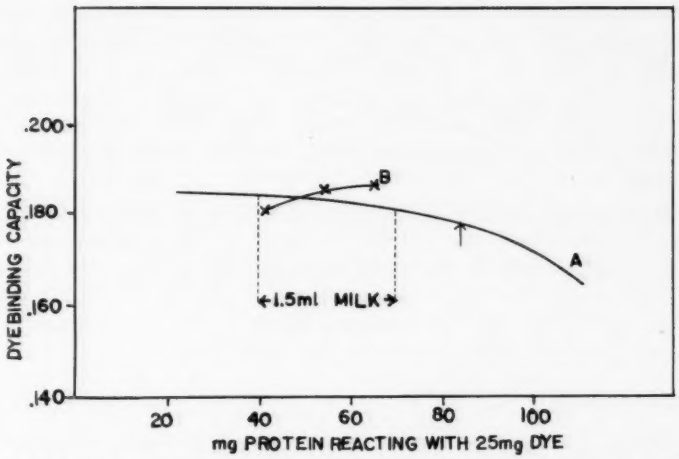


FIG. 3. Relation between the amount of dye bound per unit weight of protein (DBC) and the amount of protein in the sample. Curve A—for increasing amounts of protein from the same source. B—mean values for individual cows' milk, grouped according to protein content (data from Table 1).

arrow at 85 mg. of protein (Figure 3) marks the upper limit of protein which could be measured. Similar curves were prepared for several different milk powders, as well as samples of isolated milk proteins (casein, whey protein). All curves showed the same downward trend, which can be explained by assuming that all dye binding sites on the protein molecule do not have the same affinity for the dye. When the protein content of the sample fell between 40 and 70 mg. no significant change in the DBC was apparent.

Curve B shows the results of calculating the mean DBC for three points on the common regression line shown in Table 1. The first point included 36 samples below 3.0% protein (mean 2.74%). The second point included 249 samples between 3.0 and 4.0% protein (mean 3.53%). The third point included 60 samples above 4.0% protein (mean 4.23%). A possible explanation for the slight increase in DBC, as the total protein increases for these milks from individual cows, may lie in the distribution of the nitrogen. As previously mentioned, whey proteins have a higher DBC than casein. Nitrogen distribution data on these samples were not available.

*Dye binding of nonprotein components of milk.*

Udy (8) had stated that the only milk constituents which bind the dye are the proteins, since an extrapolated straight line relating protein percentage and the concentration of unbound dye ran through the point of zero per cent protein and the original dye concentration. This assumption is not safe because of the extended extrapolation.

Several findings in the present experiment tend to confirm Udy's (8) assumption: (a) DBC values for whole milk powder and nonfat milk powder from the

same lots of whole milk are the same; (b) 1 ml. of butterfat from which the curd had been removed by washing with hot water bound no measurable amount of dye; (c) when 0.5 ml. of the butterfat was added to 1 ml. of skimmilk containing 35.0 mg. of protein ($N \times 6.38$), the dye binding procedure showed 34.8 mg. of protein; and (d) increasing amounts of lactose up to as much as 85 mg. could be added to 65 mg. of pure casein sol in a 2-ml. volume without affecting the DBC of the casein (4).

Additional proof that the dye binding of protein is independent of the fat content was secured by comparing the fat content of the milk samples shown in Table 1 with the error of the dye binding method. This error was calculated as the difference between results for protein found by the macro-Kjeldahl procedure and results secured by the dye binding procedure. There was no relationship between the size of the error and the per cent fat in the milk.

*Use of preservatives.*

The influence of several preservatives on the dye binding results has been investigated. As might be expected, low temperature storage proved to be the best means of preserving the milk samples. Hydrogen peroxide has been used routinely in this laboratory as an additional precaution. Two drops of 15% $H_2O_2$ per 100 ml. of milk had no effect on the percentage protein found. At room temperature this preservative was effective for not more than two days.

The use of formaldehyde lowered the percentage of protein when the milk was stored at room temperature. The extent of the lowering depended not only on the amount of formaldehyde added but also on the length of storage and the temperature. Two drops of 38% HCHO solution added to 100 ml. of milk lowered the per cent protein by about 0.2% after 1 wk. of storage at room temperature (a 5% loss of protein).

A loss of protein was noted also when 125 mg. of potassium dichromate was used per 100 ml. of milk. At first, higher protein values were found. After 1 wk. of storage at room temperature the preserved samples were lower than the controls stored in the refrigerator.

Mercuric chloride appears to be the best preservative to use for room temperature storage; 135 mg. of reagent grade $HgCl_2$ preserved 100 ml. of milk for 1 wk., with a loss of about 0.1% protein. Controls stored just above freezing showed about the same loss of protein as measured by dye binding after 1 wk. of storage. Steinholtz (5) used this preservative with success also.

Several antibiotics were tried and none proved satisfactory as a preservative for more than a few days. In general, the measured amount of protein remained fairly constant until coagulation occurred.

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# STUDIES ON MILK POWDERS. IV. THE FOAM AND SEDIMENT FRACTIONS OF RECONSTITUTED WHOLE MILK [1]

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**SUMMARY**

Whole milk powder was reconstituted and the resultant whole milk was passed through a 200-mesh sieve. This treatment removed virtually all the foam or scum from the surface of the milk. The milk was then centrifuged. The insoluble material (foam fraction) which remained on the sieve, and the insoluble material (sediment fraction) which was recovered by centrifugation, were washed with water and freeze-dried. The dried foam fraction contained about 50% more fat than did the sediment fraction. Melting point and iodine value determinations on the ether-extractable portions of the foam and sediment indicated that the fat of the sediment contained a higher proportion of unsaturated fatty acids than did the fat of the foam. Results of electrophoretic analyses of the ether-insoluble portions of the foam and sediment indicated that the major protein constituent of the sediment was beta-lactoglobulin, and that of the foam was casein.

Reconstituted whole milk usually contains insoluble material. Some of this insoluble material may be observed as a scum at the surface and as a sediment which deposits upon low-speed centrifugation of the milk. Wilster, Schreiter, and Tracy (13) investigated some factors which may affect the formation of scum on the surface of reconstituted whole milk. They found that certain conditions of manufacture, such as the homogenization of the milk prior to drying and avoidance of overheating of the powder in the drying chamber, tended to reduce scum formation.

Waite and White (11) concluded from their studies on three fractions obtained by the centrifugation of reconstituted whole milk, that when considerable insolubility exists, the protein is carried into the fat layer and the fat into the sediment layer in quantities directly related to the degree of insolubility of the milk powder.

Litman and Ashworth (7) have shown that considerable scum formed on milk powder stored at 85° F., but that little scum formed when the powder was held at 45° F. Scum seemed to be related to the initial free-fat content of the powder. It was suggested that the development of scum is the result of formation of a fat-protein complex involving free-fat and unstable protein, with possible involvement of calcium. Fat isolated from scum had a higher melting point and was more saturated than the fat that remained in the scum-free fraction of the milk.

Julien and Baker (6) studied the composition of insoluble material in reconstituted whole milk. They observed that the foam fraction which collected at the

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surface of the milk contained more than twice as much butterfat as did the sediment which was recovered by low-speed centrifugation. Measurement of fat indices of the ether extracts of the two fractions showed that their fat composition was different.

The foam or scum which may be observed on the surface of reconstituted whole milk, and which coats the sides of containers, gives the milk a displeasing appearance. The present work was undertaken in the hope that more precise information concerning the composition of this material would suggest changes in the conditions of manufacture of whole milk powder, which would prevent scum formation in the reconstituted milk.

## MATERIALS AND METHODS

Freshly prepared commercial whole milk powder was vacuum-packed and stored at 25° C. for 2 wk. The method of manufacture of the milk powder was as follows: Whole milk was standardized at 3.3% butterfat and was then preheated at 200–210° C. by means of a flash plate heater. The milk was condensed to 40% solids in a triple-effect vacuum pan and was then spray-dried in a Rodger's Spray Drier. The milk powder (butterfat 28.0%; moisture 1.5%), after storage for 2 wk., had a solubility index (ADMI) of 0.1.

*Free fatty acids.* A mixture of 1 g. of fat and 10 ml. of neutral ethanol was heated to boiling and quickly titrated with 0.1 *N* sodium hydroxide.

*Saponification number.* After titration of the free fatty acids, potassium hydroxide was added to the flask and the mixture was refluxed for 30 min., then cooled. The soap solution was titrated to the phenolphthalein end point with 0.5 *N* hydrochloric acid.

*Volatile water-soluble fatty acids.* The soap solution which was obtained from the saponification number determination was diluted to 100 ml., and 10 ml. of this solution was transferred to a distillation flask recommended by Friedmann (5), along with 50% sulfuric acid. The mixture was steam-distilled and the distillate was filtered and then titrated to the phenolphthalein end point with 0.01 *N* sodium hydroxide.

*Determination of fatty acids.* The samples were interesterified with methanol and were then gas-chromatographed by means of a butanediol succinic acid column (6 ft.) and a diethylene glycol succinic acid column (4 ft.). The columns were operated at 200° C. and at a flow (helium) rate of 120 cc. per minute.

*Isolation of foam and sediment fractions.* Twenty pounds of powder were reconstituted in 13-g. lots according to the A.D.M.I. Method for the determination of solubility index, and the milk was passed through a 200-mesh sieve. This removed virtually all of the foam. After the passage of each 500-ml. portion of reconstituted milk through the sieve, the sieve was back-washed with 400 ml. of water and the resulting mixture was stirred for a few minutes and then allowed to stand overnight at 5° C. The floating layer was collected from the surface by means of a spatula. The sediment fraction which was investigated was that obtained by the A.D.M.I. method for the determination of solubility index. The floating layer (foam) and sediment fraction, which were obtained from 20 lb. of

milk powder, were freeze-dried and then stored over phosphorus pentoxide in vacuo. The yields of foam and sediment were 3.1 and 5.0 g., respectively, per kilogram of powder. The two materials were analyzed for free fat (by extraction with ethyl ether in a Soxhlet fat extractor for 18 hr.) and for ash (by heating the sample in a porcelain crucible at 550° C. for 5 hr.). The following determinations were carried out in duplicate on the free fat as well as on a sample of butter oil prepared from the same milk as had been used to prepare the powder: Melting point, free fatty acid, saponification number, iodine value (1), and volatile water-soluble fatty acids. The following determinations were carried out on the non-extractable portions of the foam and of the sediment: Nitrogen (1), ash, fat by the Roese-Gottlieb method (1), carbohydrate by the method of Reynolds *et al.* (9), and moisture (1). The ash obtained by ignition of the nonextractable portions of the foam and of the sediment was analyzed for calcium by the standard ammonium oxalate method (1), for phosphorus by the method of Dickman and Bray (4), and for potassium by flame photometry (3). The nitrogen distributions of the nonextractable portions of the foam and of the sediment were determined according to the method of Shahani and Sommer (10).

## RESULTS AND DISCUSSION

The results reported in Table 1 show that the fat content of both fractions (foam, sediment) was much higher than the fat content of the original powder (28%). The sediment contained about the same amount of fat as the scum fraction described by Litman and Ashworth (7), and somewhat more than half as

TABLE 1
Composition of foam and sediment fractions

| Determination | Foam fraction solids | | Sediment fraction solids | | Butterfat |
|---|---|---|---|---|---|
| Ether-extractable (%) | | 79.7 | | 52.5 | |
| Saponification No. | 226 | | 215 | | 235 |
| Free fatty acids (expressed as oleic acid) (%) | 0.56 | | 0.54 | | 0.42 |
| Iodine No. | 40.6 | | 46.4 | | 35.0 |
| Volatile water-soluble fatty acid | 37.8 | | 38.5 | | 31.0 |
| Melting range *(° C.)* | 29–35 | | 15–30 | | 33–35 |
| Nonextractable *(%)* | | 21.7 | | 47.5 | |
| Total nitrogen *(%)* | 9.78 | | 11.6 | | |
| Protein ($N \times 6.38$) *(%)* | 62.4 | | 74.5 | | |
| Noncasein + nondenatured whey protein N | 4.57 | | 3.20 | | |
| Proteose peptone + NPN | 4.39 | | 2.96 | | |
| NPN | 1.10 | | 0.57 | | |
| Globulin N | 0.94 | | 1.04 | | |
| Casein + denatured whey protein N (by diff.) | 5.21 | | 8.47 | | |
| Fat (Roese-Gottlieb) (%) | 4.2 | | 5.0 | | |
| Carbohydrate *(%)* | 14.2 | | 3.4 | | |
| Moisture *(%)* | 8.2 | | 8.2 | | |
| Ash *(%)* | | 6.9 | | 7.0 | |
| Calcium *(% of ash)* | 27.5 | | 34.2 | | |
| Potassium *(% of ash)* | 7.29 | | 1.25 | | |
| Phosphorus *(% of ash)* | 19.8 | | 21.5 | | |

much fat as the foam fraction described in the present paper. The saponification number of the foam-fat and the sediment-fat are both lower than that of butterfat of the powder. The higher proportion of unsaturated fatty acids and of low molecular weight fatty acids (Table 2) in the sediment-fat would account for the lower melting range of this material.

The results of the determinations of nitrogen distribution show that the ether-insoluble portion of the foam contained more proteose-peptone nitrogen than did the ether-insoluble portion of the sediment. The higher proteose-peptone nitrogen and nonprotein nitrogen may have been due to the fact that the sediment was washed more thoroughly than the foam. The higher carbohydrate content of the ether-insoluble portion of the foam may be explicable on the same grounds.

It was of interest to know whether the casein and denatured whey protein fractions of the foam and sediment were similar in composition. To check this point, the two products (ether-insoluble portions of foam and sediment) were dissolved in 0.1 *N* sodium hydroxide. Zone electrophoresis (8) was performed on each of the two solutions, as well as on a sample of Warner casein (12) and beta-lactoglobulin (Bovine, 3×, Nutritional Biochemicals, Cleveland 28, Ohio). Photographs of the electropherograms are shown in Figure 1. The two

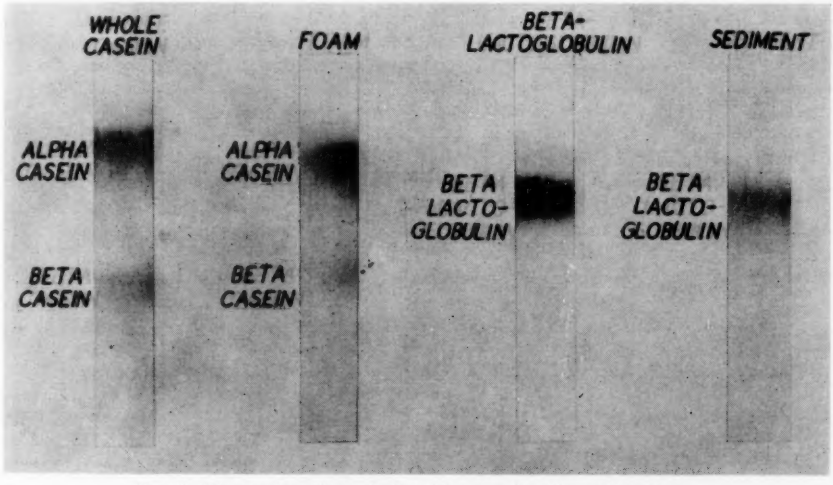


FIG. 1. Electrophoretic analysis of foam and sediment fractions.

deeply stained bands which appeared on the electropherograms of the foam protein behaved electrophoretically in the same way as alpha- and beta-casein. The single deeply stained band which appeared on the electropherogram of the sediment behaved in the same way as the beta-lactoglobulin. These results provide evidence that the main protein component of the foam fraction is casein and that the major protein component of the sediment fraction is beta-lactoglobulin.

Baker *et al.* (2) fractionated butter oil to obtain products melting at 19–21,

TABLE 2

Composition of the ether-soluble portion of foam and sediment [a]

| | $C_4$ | $C_6$ | $C_8$ | $C_{10}$ | $C_{11}$ | $C_{12}$ | $C_{12}^{=}$ | $C_{13}$ | $C_{14}^{iso}$ | $C_{14}$ | $C_{14}^{=}$ | $C_{15}$ | $C_{16}^{iso}$ | $C_{16}$ | $C_{16}^{=}$ | $C_{17}$ | $C_{18}^{iso}$ | $C_{18}$ | $C_{18}^{1=}$ | $C_{18}^{2=}$ | $C_{18}^{3=}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Foam | 0.7 | 1.4 | 1.3 | 3.1 | 0.3 | 3.1 | T. | T. | T. | 10.6 | 2.0 | 1.6 | T. | 25.6 | 3.0 | 1.2 | T. | 13.5 | 28.0 | 2.6 | 1.9 |
| Sediment | 1.2 | 2.2 | 2.1 | 3.1 | 0.3 | 2.8 | T. | T. | T. | 9.8 | 1.9 | 1.9 | T. | 24.5 | 3.2 | 0.8 | T. | 12.3 | 28.9 | 2.6 | 2.2 |
| Butterfat | 1.1 | 1.7 | 1.4 | 3.0 | 0.4 | 3.7 | — | T. | T. | 11.9 | 1.6 | 1.6 | 0.4 | 31.0 | 3.0 | 0.8 | — | 11.2 | 24.0 | 1.7 | 1.5 |

[a] The data are expressed in weight per cent of total eluate. The subscript below C shows the number of carbon atoms in the straight acids. The exact configuration of the carbon skeleton of the iso-compounds is not known. Two horizontal bars above the C indicate a double bond, and the number refers to the number of double bonds. T. indicates trace.

22–24, and 28–30° C. and prepared agglomerated milk powders, each containing one of the fat fractions. It has been observed in the course of the present work that scum was not present on the milk reconstituted from the powder that contained the low-melting fraction (19–21° C.). This would suggest that the higher-melting triglycerides play an important role in scum formation.

ACKNOWLEDGMENTS

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# SOME BIOCHEMICAL CONSTITUENTS IN SERUM, CEREBROSPINAL FLUID, AND AQUEOUS HUMOR OF VITAMIN A DEFICIENT HOLSTEIN CALVES [1]

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## SUMMARY

Sixteen male Holstein calves previously raised to 63 days of age on a limited whole milk, limited starter, and ad libitum chopped alfalfa hay ration were fed a vitamin A depletion ration until their plasma vitamin A averaged 8.3 $\pm$ 2.2 $\gamma$ per 100 ml. Each calf was then fed for a 16-wk. period one of four carotene intakes, 16 or 24 $\gamma$ per pound of live weight per day, to provide marked increases in cerebrospinal fluid pressure, 32 $\gamma$, to provide slight elevation in pressure, or 40 $\gamma$, to provide no elevation. Terminal cerebrospinal fluid pressure decreased with increasing intakes of carotene, apparent intraocular (scleral) pressure exhibited no consistent change, and plasma and liver concentrations of carotenoids and vitamin A increased. Of the biochemical constituents studied, no trends with carotene intake were observed for serum, and only potassium of cerebrospinal fluid decreased slightly; whereas, for aqueous humor, sodium was higher in the calves fed the 16-, 24-, and 32-$\gamma$ carotene intakes, potassium increased with an increase in carotene intake, chloride decreased, and osmotic pressure was greater in calves fed the 16-, 24-, and 32-$\gamma$ carotene intakes than in those fed the 40-$\gamma$ intake. These results do not directly explain the increased cerebrospinal fluid pressure or change in aqueous humor composition occurring in vitamin A deficiency. However, the data pertaining to cerebrospinal fluid suggest either an overproduction or underabsorption of fluid (without change in the concentration of the constituents in serum or cerebrospinal fluid), resulting in greater volume within the restricted subarachnoid space and ventricles of the central nervous system.

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The specific metabolic function of vitamin A at the molecular level, aside from its role in vision, has so far eluded the efforts of many investigators, T. Moore (32). Although definite changes in certain epithelial tissues, bones, and teeth are observed in avitaminosis A, Follis (19), little is known as to the role of vitamin A in preventing or alleviating these changes. Since the initial studies of L. A. Moore and J. F. Sykes (30, 31), it has become increasingly evident that the first measurable change which occurs in experimentally produced vitamin A deficiency in the calf (33), chick (40), rabbit (28), and pig (38) is an increase in the cerebrospinal fluid pressure, rather than the classical changes cited above.

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Davson (10) has stated that both cerebrospinal fluid and aqueous humor are formed by mechanisms that have a great deal in common. He also gave evidence of the positive association between cerebrospinal fluid pressure and retinal venous pressure, and discussed the possible elevation of intraocular pressure due to increased venous retinal pressure. At the initiation of this project in the spring of 1958, no reports were found in the literature by the authors of intraocular pressure measurements on vitamin A deficient animals. However, such had been suggested by Moore and Sykes (30), as well as by McLaren (26). Subsequently, in a pilot study (12) at this station with four pairs of calves, the vitamin A deficient calves of each pair were observed to average 3.4 mm. of Hg greater in intraocular pressure, measured in the scleral region, than the vitamin A adequate calves.

The cause(s) of increased cerebrospinal fluid pressure in vitamin A deficient animals is not known. One possible explanation is that the increased pressure is due to faulty and excessive bone growth, particularly of the skull bones which results in constriction of and eventual severing of the optic nerve (6). However, this does not seem tenable, for in mature cows fed a ration deficient in vitamin A (29) papilledema is present, which is preceded or accompanied by increased cerebrospinal fluid pressure, but not blindness due to constriction of the optic nerve. Also, the rather rapid decrease in cerebrospinal fluid pressures in vitamin A deficient animals (21, 27, 38), upon subsequent administration of vitamin A, strongly suggests that bone growth per se is not a causative factor of the increased pressure. Another explanation is that the increased cerebrospinal fluid pressure is due to an overproduction of the fluid by the choroid plexus, Millen and Dickson (27), and/or an obstruction at the sites of absorption of the fluid, both causing greater volume of fluid in the relatively restricted subarachnoid space and ventricles of the central nervous system. However, little has been accomplished in vigorously testing these hypotheses, because data with respect to the measurement of formation and absorption of cerebrospinal fluid are extremely difficult to interpret (35, 41).

Overproduction and/or underabsorption of cerebrospinal fluid as discussed above does not necessitate a concomitant change(s) in the concentration of constituents of cerebrospinal fluid or in blood. If there is sufficient change in the concentration of the constituents of blood such that its osmotic pressure decreases, then it would be anticipated that this change would be accompanied by increased cerebrospinal fluid pressure, Davson (10), due to the transfer of water across the blood-cerebrospinal barrier. While the present authors were unable to find literature with respect to both blood and cerebrospinal fluid composition of vitamin A deficient animals, some reports have been made with respect to blood. Moore and Sykes (31) in 1941 stated that "colloidal osmotic pressure measurements of blood plasma and various blood and urine analysis have not shown any abnormality which could be related to the raised pressure." Madsen and Earle (25), in an extensive study of field and experimental cases of hypovitaminosis A of beef cattle, reported in 1947 decreases in plasma albumin concentration, increases in total plasma globulin due primarily to marked increases in plasma

fibrinogen, and slight decreases in serum calcium and inorganic phosphorous. Calculations indicated that the decrease in the colloid osmotic pressure of the plasma due to the decrease in albumin was largely offset by the increase in the other protein fractions. More recently, Erwin *et al.* (16) found a decreased percentage of albumin in vitamin A deficiency of steers and in one pair of identical-twin Holstein heifers (17) which was largely alleviated upon administration of carotene in the first study and vitamin A in the second. In the aforementioned pilot study conducted at this station (12), slight increases in total serum protein attributable primarily to the gamma-globulin fraction, were observed in the deficient calves. Also, cerebrospinal fluid total protein increased, but inappreciable change occurred in concentrations of sodium, potassium, inorganic phosphorous and calcium and osmotic pressure as determined by cryoscopy.

The primary objective of the present study was to determine several biochemical constituents in serum, cerebrospinal fluid, and aqueous humor of Holstein calves fed graded levels of carotene so as to produce marked, slight, and no increases in cerebrospinal pressure; secondly, to determine the possible effects of varying carotene intakes on intraocular pressure and, finally, to ascertain possible association between physiological changes and changes in biochemical constituents.

## EXPERIMENTAL PROCEDURE

*Animals and feeding.* Sixteen, one-day-old male Holstein calves obtained during the period August-October, 1958, from various state institution herds, were brought to the research barn and placed in individual tie stalls. Upon arrival each calf received orally 1–500 mg. oblet of chlortetracycline (Lederle's Aureomycin) and one gelatin capsule containing 100,000 U.S.P. units of vitamin A (Nopcay "250", Type M containing 250,000 U.S.P. units per gram). If the calf had not nursed, it received 4.0 lb. of colostrum from a frozen colostrum bank for two successive feedings. Each calf was then raised to approximately its 63rd day of age on a limited whole milk, limited vitamin A depletion grain, and ad libitum chopped alfalfa hay ration as previously described (15), except that artificially dehydrated alfalfa was added to the depletion grain at the rate of 5 lb. to 100 lb. of depletion grain so as to provide sufficient dietary carotene.

On approximately the 64th day of age, each calf received a vitamin A depletion ration, two-thirds depletion grain and one-third dried beet pulp, such that an increase in live weight of 10 lb. per week would be anticipated. When the blood plasma vitamin A concentration, based on blood samples taken at successive seven-day periods, had decreased to equal to or less than 12.0 $\gamma$ per 100 ml., each calf was fed, after the next seven-day period, one of four carotene intakes, 16, 24, 32, or 40 $\gamma$ per pound of live weight per day from artificially dehydrated alfalfa meal. The 16- and 24-$\gamma$ levels of carotene intake were chosen on the basis of previous data (33) to provide insufficient carotene resulting in increases in cerebrospinal fluid pressure, 32 $\gamma$ to supply slightly above borderline intake resulting in inappreciable increases in cerebrospinal fluid pressure, and 40 $\gamma$ to provide ample carotene intake. Assignment of the calves to their respective carotene intakes was random in blocks of four calves each, with the first four

to arrive at the research barn comprising the first block, and so forth. Average age at the beginning of the carotene supplementation period was 78 days, with a standard deviation of four. Upon completion of 16 consecutive seven-day periods of supplementation with carotene, each calf was slaughtered.

Procedures followed in calculation of alfalfa to be fed to furnish the indicated carotene intake, mixing of the alfalfa into the vitamin A depletion ration, and treatment for scours were identical to those previously reported (33). Average daily minimum and maximum temperatures during carotene supplementation were 56.1 and 62.6° F., with their respective standard errors 0.2 and 0.2. Average artificial light intensity to which the calves were exposed from 6 A.M. to 6 P.M. daily, during supplementation with carotene and measured approximately at a height of 4 ft. in the center of each stall, was $7.9 \pm 0.7$ foot-candles.

*Observations.* With the exception of the artificially dehydrated alfalfa meal, all feeds fed and refused were weighed to the nearest 0.1 lb. Artificially dehydrated alfalfa was weighed to the nearest 0.1 g. Live weights were taken at successive seven-day periods throughout the experiment and linear growth measurements at the beginning and termination of supplementation with carotene.

Thirty-milliliter blood samples were obtained by jugular puncture at successive seven-day periods during vitamin A depletion, the period immediately prior to carotene supplementation, and during 28-day periods thereafter for plasma carotenoid and vitamin A analyses. On the fourth day prior to slaughter, 60 ml. of blood was withdrawn for preparation of serum for subsequent analyses for protein and minerals and for measurement of osmotic pressure by cryoscopy.

Cerebrospinal fluid pressures, in millimeters of saline, were measured ten days prior to slaughter by puncture of the subarachnoid space through the dorsal opening in the atlanto-occipital articulation, with an 18-gauge, 90-mm. length (to the base of the hub) spinal (Quincke) stainless steel needle. The needle was connected to a saline (0.85 g. NaCl/100 ml.) manometer by polyethylene tubing, both 2.0-mm. bore, and the pressures measured according to procedures as outlined by Sykes and Moore (39). Three days prior to slaughter another cerebrospinal puncture was made and approximately 40 to 60 ml. of fluid was removed with a glass syringe for subsequent analyses.

Apparent intraocular pressures four days prior to carotene supplementation and five days prior to slaughter were recorded by restraining the calf on a table. The eyelids were then held open and a topical anaesthetic, approximately 1 ml. of a 1.0% solution of cocaine hydrochloride, applied directly on the eyeball. An electronic tonometer (Model OP-9037, V. Mueller and Co.) with a 5.5-gram plunger in the tonometer head was used to record the intraocular pressure. All readings were made in the scleral region adjacent to the cornea.

Electrocardiograms were taken the week prior to slaughter after clipping both forelegs and the left hind leg, above the knees and hock, respectively, and placing the calf in a wood metabolism crate. The three standard limb leads (14) were secured after electrode paste had been applied. The calf was allowed to remain in the crate, with electrodes attached, until calm, and the electrocardiogram was then recorded, using a Sanborn Viso-Cardiette, Model 51.

At slaughter, both eyes were immediately removed, taken to the laboratory, and the aqueous humor obtained by puncture of the cornea with a scalpel, and the fluid from both combined for immediate analyses for protein, minerals, and osmotic pressure measurements. The eyes were fixed in formalin and subsequently examined for the presence or absence of papillary edema. The liver was removed, ground, sampled, and the samples were held at 0° F. for subsequent carotenoid and vitamin A analyses.

*Analyses.* Samples of all feeds fed were analyzed for proximate constituents at 4-wk. periods by A.O.A.C. methods (4), Table 1. The artificially dehydrated alfalfa meal was analyzed for carotene periodically during the course of the experiment by the A.O.A.C. procedure (4), which employed extraction overnight at room temperature instead of Soxhlet extraction.

Carotenoids and vitamin A concentrations in the plasma were determined by the Kimble procedure (23) and in the liver by a modification of the Gallup-Hoefer method (8).

Protein fractionation of serum and cerebrospinal fluid was carried out with a Spinco (Model R, Series D) paper electrophoresis system, using a barbiturate buffer consisting of 2.76 g. diethylbarbituric acid, 15.45 g. sodium barbital, and 2.0 ml. "Sterox" per 1,000 ml. (13), pH 8.6, and ionic strength 0.075, and employing a current of 5 ma. for 16 hr., all procedures essentially as described by Block, Durrum, and Zweig (7). The rapid bromphenol blue procedure (7) was used in dyeing the proteins and a Spinco Analytrol (Model RB) was used in quantitation. Prior to protein fractionation of cerebrospinal fluid, between 15 and 30 ml. were dialyzed against polyvinylpyrrolidone (Plasdone, commercial, General Aniline and Film Corp., N. Y.) as described by Lemmen *et al.* (24). Upon completion of dialysis, the bottom portion of the dialysis sack was washed with 250 μl. of the barbiturate buffer and 12 to 20 μl. of this applied to the filter paper strips. The amount applied to the filter paper strips was determined by consideration of the total protein concentration of cerebrospinal fluid and of serum, so that approximately equivalent amounts of protein from both sources were used in their respective electrophoretic separations. Total protein concentration of serum, cerebrospinal fluid, and aqueous humor was determined by the biuret reaction, Gornall *et al.* (20).

The calcium content of serum and cerebrospinal fluid was determined by flame photometry (11), the inorganic phosphorous concentration of serum, cerebrospinal fluid, and aqueous humor determined by the Fiske and Subbarow method (18), and sodium and potassium contents of the same three body fluids by flame photometry (2). Chloride contents were determined colorimetrically by the procedures of Barney and Bertolacini (5).

Osmotic pressure of serum, cerebrospinal fluid, and aqueous humor was determined, using a Model D, Fiske Osmometer (3). By definition, one milliosmol is equivalent to a freezing point depression of 0.00186° C.

Statistical procedures as set forth by Cochran and Cox (9) and Snedecor (37) were used. The analysis of variance consisted of isolating variability due to blocks of calves, among carotene intakes and remainder (error). Prior to the

TABLE 1

Average chemical composition of feeds

| | Per cent dry matter | Per cent of dry matter | | | | | |
|---|---|---|---|---|---|---|---|
| | | Crude protein | Ether extract | Crude fiber | N.F.E. | Ash | Carotene |
| | | | | | | | *(mg/lb)* |
| Alfalfa hay chopped | 90.6 ± 1.0[a] | 19.4 ± 1.8 | 2.6 ± 0.3 | 32.2 ± 2.5 | 38.1 ± 1.4 | 7.7 ± 0.7 | 3.7 ± 0.4 |
| Depletion grain | 89.8 ± 0.7 | 19.7 ± 0.7 | 4.4 ± 0.2 | 7.5 ± 0.2 | 61.7 ± 0.7 | 6.7 ± 0.2 | 0.8 ± 0.3 |
| Vitamin A depletion ration | 90.6 ± 0.5 | 16.2 ± 0.4 | 2.7 ± 0.3 | 12.0 ± 0.3 | 62.5 ± 0.4 | 6.6 ± 0.3 | 0.2 ± 0.1 |
| Artificially dehydrated alfalfa meal | 90.8 ± 1.0 | 24.0 ± 0.5 | 4.3 ± 0.2 | 19.7 ± 0.3 | 39.8 ± 0.8 | 12.2 ± 0.1 | 98.5 ± 1.1 |

[a] Standard error of mean.

start of the experiment, the among carotene intakes variability was further subdivided into two sets of single degree of freedom orthogonal contrasts as follows: linear, quadratic, and cubic trends of response on carotene intake, between the 40-γ intake and the 16- plus 24- plus 32-γ intakes, and the linear and quadratic responses on the 16-, 24-, and 32-γ carotene intakes. The reasoning which led to these comparisons was that a trend in the responses might exist across all carotene intakes or, possibly, exist only in the three lowest carotene intake responses in which slight to markedly increased cerebrospinal fluid pressures were anticipated, based on past experience (33). The transformation of the liver vitamin A concentrations to logarithms and the functional form of the responses of both plasma vitamin A and the logarithm of liver vitamin A concentrations on log carotene intake have been dealt with previously (34). A physical injury resulted in the inability of one calf, in the fourth block fed the 16-γ carotene intake, to adduct its left hind limb and subsequently caused atrophy of the muscles of the affected limb. Another calf, in the fourth block fed the 40-γ carotene intake, threw and injured itself while a cerebrospinal puncture was made. Subsequent punctures resulted in only cloudy cerebrospinal fluid being obtained. In the case of the first-mentioned calf, missing values were calculated (9) for all criteria and in the case of the second, cerebrospinal fluid and aqueous humor constituents criteria.

## RESULTS

*Feed consumption, growth, and health.* In general, calves readily consumed their vitamin A depletion ration allowance, but there was a slight tendency for increased feed refusals with decreasing carotene intakes from the 40- to the 16-γ level. This was evidenced by a significant ($P < 0.05$) negative linear trend in the transformed percentage of per cent days consuming vitamin A depletion ration allowance values, as presented in Table 2.

Growth of the calves was inappreciably affected by carotene intake. The average values prior to carotene supplementation were for live weight 184 lb., height at withers 34.2 in., heart girth 36.7 in., and girth of paunch 43.5 in., with their respective standard deviations being 16, 0.7, 1.2, and 1.8. Increases during the 16-wk. carotene supplementation period with standard deviations were, respectively, live weight 227 and 8, height at withers 7.4 and 0.9, heart girth 12.3 and 0.9, and girth of paunch 15.6 and 2.3.

There were no particular health problems encountered during the course of the experiment, with the exception of the two calves previously mentioned in the last portion of the Experimental section. Per cent days free from scours during carotene supplementation averaged 99.7. These percentage values showed no trend with carotene intake which was confirmed upon analysis of variance of the per cent days free from scours after applying the arc sin transformation. Since the occurrence of scours was never accompanied by rectal temperatures equal to or greater than 103° F., no calves required treatment for scours.

*Cerebrospinal fluid and intraocular (scleral) pressures, papillary edema, electrocardiograms, carotenoid and vitamin A concentrations.* As the carotene intake was decreased from 40- to the 16-γ level, cerebrospinal fluid pressures increased,

TABLE 2

Effect of carotene intake on feed consumption, cerebrospinal and intraocular (scleral) pressures, and carotenoid and vitamin A concentrations of Holstein male calves

| | Carotene intake (γ/lb live weight/day)[a] | | | | Standard deviation per calf |
|---|---|---|---|---|---|
| | 16 | 24 | 32 | 40 | |
| Av. per cent days consuming ration allowance | 97.9(83)[b] | 99.6(88) | 100.0(90) | 99.8(89) | — (4) |
| Terminal cerebrospinal fluid pressure *(mm. saline)* | | | | | |
| Actual | 172 | 175 | 131 | 44 | — |
| Log | 2.21 | 2.24 | 2.10 | 1.64 | 0.12 |
| Apparent intraocular (scleral) pressure *(mm. Hg)* | | | | | |
| Initial | 21.2 | 17.0 | 20.5 | 19.5 | 3.6 |
| Terminal | 23.4 | 24.8 | 22.6 | 23.1 | 3.7 |
| Terminal papillary edema *(No. of calves)* | 2 | 3 | 2 | 0 | — |
| Plasma carotenoids *(γ/100 ml.)* | | | | | |
| Initial | 18 | 17 | 18 | 13 | 8 |
| Terminal | 18 | 24 | 32 | 28 | 4 |
| Plasma vitamin A *(γ/100 ml.)* | | | | | |
| Initial | 10.5 | 7.6 | 8.1 | 7.0 | 2.2 |
| Terminal | 8.0 | 8.1 | 11.4 | 13.4 | 1.7 |
| Liver | | | | | |
| Weight *(g.)* | 2,699 | 2,694 | 3,024 | 2,898 | 195 |
| Carotenoids *(γ/100 g.)* | | | | | |
| Actual | 21 | 37 | 41 | 46 | — |
| Log | 1.36 | 1.56 | 1.61 | 1.65 | 0.09 |
| Vitamin A *(γ/100 g.)* | | | | | |
| Actual | 11 | 34 | 65 | 77 | — |
| Log | 1.05 | 1.49 | 1.77 | 1.84 | 0.23 |

[a] Values represent four calves per carotene intake group, except that the 16 γ group represents three calves, plus calculated missing value according to procedures for a randomized block design (9).

[b] Arc sin $\sqrt{Y}$, where Y equals per cent days consuming vitamin A depletion ration allowance.

Table 2. Since the variances of the cerebrospinal fluid pressures for a particular carotene intake group increased as the magnitude of the average pressure for the group increased, these values were transformed to logarithms. When thus expressed, the log of the cerebrospinal fluid pressure in millimeters of saline decreased $1.271 \pm 0.017$ units per log of carotene intake per pound of live weight per day, the simple correlation, r, between these two variables being 0.86.

Apparent intraocular pressures, Table 2, measured in the scleral region, were slightly higher for those calves receiving the 16- to 32-γ carotene intakes than those receiving the 40-γ carotene intake, 23.6 versus 23.1 mm. Hg. However, the difference between the two was small compared with the standard deviations per calf (calf plus measurement variability), so that the difference was not statistically significant. The average apparent intraocular pressure increased by 4.0 mm. Hg (23.5 minus 19.5) from the start of carotene supplementation to its termination, 16 wk. later.

The volume of aqueous humor from both eyes averaged for all calves 2.7 ml., with a standard deviation of 0.1. Level of carotene intake had inappreciable effect on this criterion.

Some of the calves in each group fed either the 16-, 24-, or 32-$\gamma$ carotene intake exhibited papillary edema at the termination of 16 wk. of carotene supplementation (Table 2); whereas, none occurred in the group fed the 40-$\gamma$ carotene intake.

Electrocardiographic intervals, in seconds, using the data from Lead II tracings obtained during the second week from termination of carotene supplementation, were unaffected by carotene intake. The average values for the PR interval with its standard deviation were, respectively, 0.17 and 0.02, for the QRS interval 0.06 and 0.01, and for the QT interval 0.31 and 0.03. Similar values for the systolic index, the ratio of the QT intervals to the square root of the time in seconds for a complete cardiac cycle (1), were 0.40 and 0.03. Average heart rate in beats per minute during electrocardiographic measurements was 98 and its standard deviation ten, and this criterion was also unaffected by carotene intake.

As might have been expected, carotenoid and vitamin A concentration of both plasma and liver, Table 2, increased with carotene intake. Plasma vitamin A increased $14 \pm 3$ $\gamma$ per 100 ml. for each log unit increase in carotene intake and, similarly, the log of the liver vitamin A increased $0.72 \pm 0.15$. The simple correlations between the respective response variables and log carotene intake were 0.91 and 0.41.

*Serum constituents.* The various biochemical constituents determined in serum, Table 3, exhibited no particular or consistent trends with change of carotene intake.

*Cerebrospinal fluid constituents.* Most of the biochemical constituents determined in cerebrospinal fluid showed little change with changes in carotene intake, Table 4. There was a tendency for those calves fed the lowest level of carotene, 16 $\gamma$, to have somewhat higher concentrations of total protein and albumin, which was also reflected in the distribution of the proteins (per cent values, Herdan's value as well as the albumin/globulin ratio). None of these differences, however, approached statistical significance. The only constituent to show a consistent change was potassium, which decreased only slightly in a linear manner ($P < 0.05$) with an increase of carotene intake.

It should be pointed out here that the errors expressed as standard deviation per calf (calf plus biochemical measurement variability) for cerebrospinal fluid were, in relation to their respective experiment means, of considerably greater magnitude than those for serum.

*Aqueous humor constituents.* In possible contrast to serum and cerebrospinal fluid, biochemical constituents of aqueous humor with the exception of total protein concentration and inorganic phosphorous, showed significant change with change in carotene intake, Table 5. Sodium concentration was on the average greater in those calves fed the 16-, 24,- and 32-$\gamma$ carotene intakes than in those fed the 40-$\gamma$ intake ($P < 0.10$). Potassium concentration was less on the 16-, 24-,

TABLE 3
Effect of carotene intake on several biochemical constituents in serum of Holstein male calves

| | Carotene intake (γ/lb live weight/day)[a] | | | | Standard deviation per calf |
|---|---|---|---|---|---|
| | 16 | 24 | 32 | 40 | |
| Protein distribution *(av. %)* | | | | | |
| Albumin | 67.9 | 69.4 | 68.7 | 68.1 | 2.6 |
| Alpha-globulin | 7.0 | 7.6 | 7.2 | 7.1 | 0.4 |
| Beta-globulin | 11.4 | 10.9 | 12.0 | 11.8 | 0.7 |
| Gamma-globulin | 13.8 | 12.0 | 12.2 | 12.9 | 1.9 |
| Herdan's value[b] | 1.40 | 1.37 | 1.38 | 1.40 | 0.07 |
| Protein concentration *(g/100 ml.)* | | | | | |
| Total | 7.52 | 6.72 | 7.18 | 7.13 | 0.29 |
| Albumin | 5.10 | 4.67 | 4.93 | 4.86 | 0.24 |
| Alpha-globulin | 0.53 | 0.51 | 0.52 | 0.51 | 0.04 |
| Beta-globulin | 0.85 | 0.73 | 0.86 | 0.84 | 0.05 |
| Gamma-globulin | 1.04 | 0.81 | 0 88 | 0.92 | 0.15 |
| Albumin/globulin ratio | 2.13 | 2.28 | 2.22 | 2.14 | 0.11 |
| Calcium *(mg/100 ml.)* | 10.6 | 10.0 | 10.4 | 10.1 | 0.4 |
| Inorganic phosphorus *(mg/100 ml.)* | 9.0 | 9.0 | 9.4 | 9.4 | 0.7 |
| Sodium *(mg/100 ml.)* | 342 | 342 | 344 | 341 | 4 |
| Potassium *(mg/100 ml.)* | 18.1 | 17.0 | 17.9 | 17.9 | 1.3 |
| Chloride *(mg/100 ml.)* | 377 | 391 | 397 | 390 | 30 |
| Osmotic pressure *(milliosmols/kg of $H_2O$)* | 300 | 302 | 303 | 300 | 4 |

[a] Values represent four calves per carotene intake group, except that the 16 γ group represents three calves plus a calculated missing value, according to procedures for a randomized block design (9).

[b] "An index of the internal mobility of the mixture of proteins and of the preponderance of abnormal constituents in it" and "calculated as the negative average logarithmic probability of the serum constituents" (22).

and 32-γ carotene intake groups than in the 40-γ ($P < 0.05$) and, in addition, potassium showed a significant positive linear trend with increases in carotene intake ($P < 0.05$). Chloride concentration was higher in those calves on the three lowest carotene intakes than in those on the highest intake ($P < 0.05$), and chloride concentration decreased linearly with increases in carotene intake ($P < 0.10$). The changes in these constituents of aqueous humor were accompanied by a greater osmotic pressure of the calves fed the 16-, 24-, and 32-γ carotene intakes than those calves fed the 40-γ carotene intake ($P < 0.10$).

The errors, expressed as standard deviation per calf (calf plus biochemical measurement variability), for aqueous humor were, in relation to their respective experiment means, of approximately the same magnitude or slightly greater than those for cerebrospinal fluid.

## DISCUSSION

The results of this limited study tentatively suggest that the increased cerebrospinal fluid pressure observed in hypovitaminotic A calves is due to an overproduction of cerebrospinal fluid, as suggested by Millen and Dickson (27), and/or an obstruction to the sites of absorption, resulting in greater volume of fluid contained within the relatively fixed subarachnoid space and ventricles of the central nervous system. Evidence for this was the lack of consistent

TABLE 4
Effect of carotene intake on several biochemical constituents in cerebrospinal fluid of Holstein male calves

| | Carotene intake (γ/lb live weight/day)[a] | | | | Standard deviation per calf |
|---|---|---|---|---|---|
| | 16 | 24 | 32 | 40 | |
| Protein distribution *(av. %)* | | | | | |
| Albumin | 60.2 | 51.4 | 54.2 | 53.2 | 6.4 |
| Alpha-globulin | 13.9 | 19.3 | 18.0 | 18.2 | 3.3 |
| Beta-globulin | 13.4 | 16.5 | 15.0 | 16.7 | 3.1 |
| Gamma-globulin | 12.4 | 12.7 | 12.8 | 11.9 | 3.0 |
| Herdan's value[b] | 1.60 | 1.76 | 1.69 | 1.72 | 0.10 |
| Protein concentration *(mg/100 ml.)* | | | | | |
| Total | 44 | 28 | 38 | 34 | 7 |
| Albumin | 26 | 14 | 21 | 18 | 5 |
| Alpha-globulin | 6 | 5 | 7 | 6 | 1 |
| Beta-globulin | 6 | 5 | 6 | 6 | 1 |
| Gamma-globulin | 6 | 4 | 5 | 4 | 2 |
| Albumin/globulin ratio | 1.52 | 1.07 | 1.25 | 1.14 | 0.29 |
| Calcium *(mg/100 ml.)* | 5.4 | 5.2 | 5.4 | 5.4 | 0.1 |
| Inorganic phosphorus *(mg/100 ml.)* | 1.7 | 1.8 | 1.9 | 1.7 | 0.1 |
| Sodium *(mg/100 ml.)* | 341 | 342 | 340 | 340 | 3 |
| Potassium *(mg/100 ml.)* | 12.9 | 12.6 | 12.5 | 12.4 | 0.2 |
| Chloride *(mg/100 ml.)* | 422 | 429 | 434 | 430 | 15 |
| Osmotic pressure *(milliosmols/kg of $H_2O$)* | 294 | 295 | 297 | 295 | 3 |

[a] Values represent four calves per carotene intake group, except that the 16 and 40 γ groups each represent three calves plus a calculated missing value, according to procedures for a randomized block design (9).

[b] "An index of the internal mobility of the mixture of proteins and of the preponderance of abnormal constituents in it" and "calculated as the negative average logarithmic probability of the serum constituents" (22).

change with carotene intake of the biochemical constituents determined for serum and almost similar findings for cerebrospinal fluid. The one exception for the latter was a slight decrease in potassium with an increase in carotene intake. Since neither serum nor cerebrospinal fluid exhibited appreciable change in hypovitaminosis A, it would seem likely that the tissues involved in the production and absorption of cerebrospinal fluid are probably involved. It is of

TABLE 5
Effect of carotene intake on several biochemical constituents in aqueous humor of Holstein male calves

| | Carotene intake (γ/lb live weight/day)[a] | | | | Standard deviation per calf |
|---|---|---|---|---|---|
| | 16 | 24 | 32 | 40 | |
| Protein concentration *(mg/100 ml.)*, total | 40 | 35 | 48 | 43 | 6 |
| Inorganic phosphorus *(mg/100 ml.)* | 4.0 | 4.0 | 4.3 | 4.0 | 0.5 |
| Sodium *(mg/100 ml.)* | 339 | 352 | 359 | 338 | 10 |
| Potassium *(mg/100 ml.)* | 17.1 | 17.7 | 17.8 | 19.1 | 1.0 |
| Chloride *(mg/100 ml.)* | 456 | 502 | 447 | 410 | 42 |
| Osmotic pressure *(milliosmols/kg of $H_2O$)* | 306 | 310 | 311 | 302 | 5 |

[a] Values represent four calves per carotene intake group, except that the 16 and 40γ groups each represent three calves plus a calculated missing value, according to procedures for a randomized block design (9).

interest that Moore and Sykes (31) could find no pathological changes of either the choroid plexus, probable site of production (10), or arachnoid villi, one of the possible sites of absorption (10), in the young bovine fed a vitamin A deficient ration. If these negative pathological findings are confirmed by further research, then other approaches, such as measurement of the rate of formation or absorption (which are difficult to interpret) are needed to test the hypothesis of overproduction or underabsorption of cerebrospinal fluid in hypovitaminosis A.

The negative findings of this study with respect to serum may at first inspection appear to contradict those of Madsen and Earle (25), Erwin *et al.* (16, 17), and Dehority *et al.* (12). Madsen and Earle studied field cases or experimentally produced vitamin A deficiency of cattle of prolonged duration, usually a year or longer, contrasted to the 16-wk. study reported herein. Therefore, duration of feeding low levels of carotene intake could have accounted for the differences between the two studies. Erwin *et al.* fed essentially a carotene-free ration to steers for about a 120-day period (16) and to a set of identical-twin heifers for a 150-day period (17), in both cases until the animal exhibited vitamin A deficiency changes. Thus, these workers were probably dealing with terminal vitamin A deficiency in which changes such as anasarca, blindness, convulsions, muscular incoordination, etc. are characteristic. In contrast, in the study reported herein no such changes were noted. While the pilot study reported previously from this station, Dehority *et al.*, was somewhat similar to the one reported herein, the calves consisted of two pairs of Guernsey and two pairs of Holstein males. Guernsey calves fed the 15-γ carotene intake did not consume their vitamin A depletion ration allowance 68% of the time during carotene supplementation and those fed the 60-γ carotene intake, 11%. Holsteins had no ration refusals. Thus, inanition and breed possibly affected the results, the former being of particular importance in differentiating specific and nonspecific deficiency changes, Follis (19). It is recognized that to adequately resolve differences among experiments such as cited above, the variables mentioned should preferably be studied in a single experiment.

The absence of any consistent trend in apparent intraocular (scleral) pressure with increasing carotene intake could have indicated that this criterion is not very sensitive as an indicator of hypovitaminosis A. A second consideration is that the measurement of apparent intraocular pressure in the scleral region, even though the sclera of domestic animals is less resistant to internal pressures than in man (36), may not reflect the pressure within the eye. Therefore, this measurement may not be comparable to those taken directly or indirectly by tonometry in the corneal region. The second consideration appears more likely.

The rather marked changes occurring in the concentration of some of the constituents of aqueous humor, in contrast to no measurable changes in the concentration of the constituents of serum and a change only in concentration of potassium of the cerebrospinal fluid constituents were of interest. These differences may be explained, in part, by considering the relative magnitude of dilution accompanying similar changes in the absolute amounts of constituents in these three fluids. Why changes in the osmotic pressure and in concentrations

of some of the constituents of aqueous humor were accompanied by no increase in intraocular pressure, as measured indirectly in the scleral region, requires confirmation either by direct manometry or tonometry in the corneal area.

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# NUTRITIONAL MUSCULAR DYSTROPHY IN CALVES. II. ADDITION OF SELENIUM AND TOCOPHEROL TO A BASAL, DYSTROPHOGENIC DIET CONTAINING COD-LIVER OIL

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## SUMMARY

Nutritional muscular dystrophy was produced experimentally in Holstein-Friesian calves. The addition of cod-liver oil to the diet caused an intensification of Zenker's degeneration in the muscles of the tocopherol-deficient calves. One p.p.m. of selenium added to the basal diet did not prevent muscular dystrophy. The addition of 200 mg. of water-dispersible *d*-alpha-tocopheryl acetate per calf per day completely prevented the development of muscular dystrophy in all calves so treated. All calves which were not fed tocopherol supplements developed Zenker's degeneration of the skeletal and tongue muscles and degeneration of the Purkinje fibers of the heart. Since blood serum magnesium levels declined progressively in all calves in the experiment, it is possible that low blood magnesium levels may work synergistically with low blood tocopherol levels to produce Purkinje fiber degeneration.

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In a previous report on nutritional muscular dystrophy in calves (27), the clinical aspects of the disease were outlined and the experimental production of muscular dystrophy in Holstein-Friesian calves was reported. The addition of 10% dried brewer's yeast or 0.85% dicalcium phosphate to the basal dystrophogenic diet did not prevent the occurrence of dystrophic lesions. The results of these experiments (27) and those of Holter, Smith, and Loosli (20), working simultaneously and independently, demonstrated that when a simplified, tocopherol-deficient diet low in unsaturated fats was fed to calves, a long feeding period was required to produce muscular dystrophy.

A more complete review of the literature is contained in the previous paper (27).

The present experiment was conducted to determine if, by the addition of cod-liver oil, the lesions of muscular dystrophy produced with the tocopherol-deficient diet would be hastened or intensified, and if the addition of selenium would prevent Zenker's degeneration under the given conditions.

Agduhr and Stenstrom (1), using an electrocardiogram, detected heart lesions in calves that had been fed cod-liver oil. Barnes, Davis, and McCay (4), however, did not detect changes in the electrocardiogram of calves which were fed good or very poor diets to which cod-liver oil was added. Their data tend to discredit the conclusions of Agduhr and Stenstrom (1). Workers at Cornell University demonstrated that cod-liver oil enhanced the production of muscular dystrophy in guinea pigs, rabbits, sheep, and goats (25). Davis and Maynard (15) found that calves were more resistant to the dystrophy-producing effects of cod-liver oil than were sheep and goats. When they added 0.7 g. of cod-liver oil per kilo-

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gram of body weight to the diet of sheep and goats, dystrophy was produced in 90 days. Of six calves fed a level of 0.7 g. of cod-liver oil per kilogram of body weight per day, four developed muscular dystrophy, one at 42 days of age and the other three at 168 to 182 days of age. Davis, Maynard, and McCay (16) were not able to characterize the toxic effect of cod-liver oil.

Blaxter, Wood, and MacDonald (8) demonstrated that 18 ml. of cod-liver oil per day would nullify the action of 50 mg. of *dl*-alpha-tocopherol per day when the latter was used for the prevention of muscular dystrophy in calves. While cod-liver oil itself contains tocopherols, the effects of the unsaturated fatty acids present nullify the action of the tocopherols present in the cod-liver oil, as well as an appreciable quantity of tocopherols that are already present in the animal body (11, 30).

Schwarz (37, 38) showed that dietary liver necrosis in rats could be prevented by adding cystine and vitamin E to their diets. He also reported that dried brewer's yeast contained an unidentified factor capable of sparing the amount of vitamin E required for the prevention of liver necrosis. Schwarz called the unidentified compound Factor 3. Schwarz and Foltz (39) reported that selenium in an organic form is an integral part of Factor 3. Inorganic selenium salts, however, were remarkably effective in protecting rats against dietary necrotic liver degeneration. Selenium will not, however, replace tocopherol for some of the deficiency changes which occur in tocopherol deficiency (13, 14).

Poultry diseases which were partially or completely prevented by adding tocopherol to the diet have also been found to be partially or completely prevented by adding selenium to the diet (34, 40).

Recent work with sheep (19, 32, 33) has indicated that a deficiency of selenium in the diet of dams is involved in the production of muscular dystrophy in lambs. The feeding of 1 p.p.m. of selenium to ewes, whose diets contained less than 0.1 p.p.m. selenium, effectively prevented the appearance of dystrophy in their lambs. McLean, Thomson, and Claxton (28) reported a growth response in six out of nine groups of lambs totalling 730, of different breeding and nutritional status when selenium was given by drench or injection.

A report of interest is that of Bunyan, Edwin, and Green (12), which demonstrated that trace elements other than selenium will, to some extent, also protect rats against dietary necrotic liver degeneration. Broberg (9) emphasized the high incidence of copper and cobalt deficiencies occurring in endemic muscular dystrophy areas in Finland. Andersson (2) demonstrated that in muscular dystrophy in rabbits, additional iodide in the diet increased the incidence and severity of Zenker's degeneration. Blaxter and Sharman (7) found hypomagnesemia to be associated with muscular dystrophy of calves in Scotland.

## EXPERIMENTAL PROCEDURE

Holstein-Friesian male calves were obtained from the Cornell University dairy herds. The experiment was begun on November 27, 1958, and concluded on April 18, 1959. The length of time the individual animals were on the test is shown in Table 1. The first four calves received were placed on the basal diet, plus

TABLE 1

Necropsy reports on calves

| Calf No. | Diet [a] | Days on test | Necropsy findings |
|---|---|---|---|
| 17 | Basal | 112 [b] | Zenker's degeneration and Purkinje fiber degeneration |
| 18 | Basal | 101 [b] | Zenker's degeneration and Purkinje fiber degeneration |
| 19 | Basal | 34 | Died. *Escherichia coli* septicemia |
| 20 | Basal | 101 [b] | Zenker's degeneration and Purkinje fiber degeneration |
| 21 | Basal plus selenium | 97 | Zenker's degeneration and Purkinje fiber degeneration |
| 22 | Basal plus selenium | 97 | Zenker's degeneration and Purkinje fiber degeneration |
| 23 | Basal plus selenium | 95 | Zenker's degeneration and Purkinje fiber degeneration |
| 24 | Basal plus selenium | 15 | Died. *E. coli* septicemia |
| 25 | Basal plus selenium | 35 | Died. *E. coli* septicemia |
| 26 | Basal plus selenium and tocopherol | 94 | No pathologic lesions |
| 27 | Basal plus selenium and tocopherol | 94 | No pathologic lesions |
| 28 | Basal plus tocopherol | 30 | Died. *E. coli* septicemia [c] |
| 29 | Basal plus tocopherol | 99 | No pathologic lesions |

[a] Cod-liver oil (30 ml.) fed daily to all calves.
[b] Biopsy of *semitendinosus* muscle positive for Zenker's degeneration on 96th day of test.
[c] Very mild Zenker's degeneration on histologic section.

cod-liver oil plus selenium, and the next two were allotted to the basal diet plus cod-liver oil and tocopherol, and the following two to the basal diet, plus cod-liver oil, tocopherol, and selenium. In addition, one of the selenium-treated calves which died was replaced.

Immediately following birth, the calves were housed in individual pens containing about 18 sq. ft. of floor space. The pens were divided by partitions 4 ft. high. Wood shavings were used as bedding.

As in the previous experiments (27), the calves were not allowed to suckle the dams. For the first two days, skimmed colostrum was fed at the rate of 5% of body weight per day, divided into two equal feedings. Vitamin A (80,000 I.U.) and vitamin D (12,000 I.U.) were given in capsule form with the first feeding of skimmed colostrum. All of the calves received 30 ml. of cod-liver oil[2] per day with the morning feed. This supplied at least 25,500 I.U. of vitamin A and 2,550 I.U. of vitamin D per day.

The treatment groups were (a) basal diet, (b) basal diet plus selenium 1 p.p.m., basal diet plus selenium, 1 p.p.m., and tocopherol, 200 I.U. water-dispersible *d*-alpha-tocopheryl acetate, and basal diet plus tocopherol, 200 I.U. water-dispersible *d*-alpha-tocopheryl acetate.

The basal diet was diluted to 12.5% solids and fed initially at 6% of body weight per day; this was increased gradually to 12% of body weight per day. The basal diet consisted of: Skimmilk (dried), 70.00; dextrose, 4.875; lard (stripped), 25.00; Tween 60, 0.125; mineral mixture, 1.75 ml. per 100 g. of diet. The mineral mixture contained in milligrams per milliliter: $MnSO_4 \cdot H_2O$, 0.5; $CuSO_4 \cdot 5H_2O$, 0.5; $CoSO_4$, 0.1; $Fe(NH_4)_3(C_6H_5O_7)_2$, 20.0; and $MgSO_4$, 60.0.

The procedure for collecting the blood samples and the analytical methods employed were the same as in the previous paper (27). Calves were bled before being placed on test, at 30-day intervals, and immediately before necropsy. In addition, serum magnesium levels were determined by the Titan-yellow method (23).

The selenium content of the basal diet was determined by activation analysis[3] and the magnesium content of the basal diet was determined using the method of Bachra, Daver, and Sobel (3).

The tocopherol content of the cod-liver oil, shavings, and basal diet was determined by the method of Bro-Rasmussen and Hjärde (10).

The skimmilk used in the basal diet, and the basal diet, contained 57.6 and 161.6 mg. of magnesium per 100 g., respectively. The basal diet contained 0.3 p.p.m. selenium and 0.03 mg. of alpha plus zeta tocopherols per 100 g. The wood shavings contained 0.33 and 0.19 mg. of alpha plus zeta, and other tocopherols per 100 g., respectively, and the cod-liver oil contained 7.28 mg. of alpha tocopherol per 100 g.

The tocopherol content of the wood shavings was not high. While the calves did consume some of these shavings, the fact that the cellulose in wood shavings

[2] Nutritional Biochemicals, Cleveland, Ohio.

[3] Made by Activation Analysis Group, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

TABLE 2
Mean blood constituents and cell counts

| Measure | Treatment groups | | | |
|---|---|---|---|---|
| | Basal | Se | Se + Vit. E | Vit. E |
| Plasma tocopherol *(γ/100 ml.)* | 104 | 112 | 687 | 544 |
| Serum | | | | |
| Phosphorus *(mg/100 ml.)* | 6.7 | 7.5 | 7.7 | 6.8 |
| Calcium *(mg/100 ml.)* | 11.4 | 11.4 | 10.9 | 11.0 |
| Magnesium *(mg/100 ml.)* | 1.5 | 2.1 | 1.6 | 1.4 |
| Sodium *(meq/l)* | 141 | 142 | 144 | 143 |
| Potassium *(meq/l)* | 5.4 | 5.8 | 5.8 | 5.5 |
| Chloride *(meq/l)* | 104 | 103 | 101 | 99 |
| Hemoglobin *(g/100 ml.)* | 12.9 | | 11.7 | 12.2 |
| White cell count *(M/Cmm)* | 9.24 | 9.16 | 10.40 | 7.04 |
| Differential cell count *(%)* | | | | |
| Lymphocytes | 41 | 45 | 53 | 61 |
| Monocytes | 2 | 4 | 2 | 3 |
| Granulocytes | 47 | 51 | 45 | 36 |

is relatively unaffected by rumen microorganisms (24, 31) casts doubt as to the significance of the tocopherol levels in wood shavings. Whether the tocopherols present in wood shavings are absorbed from the rumen is, however, not known.

## RESULTS

The experimental findings are summarized in Tables 1 and 2. These data show that the plasma tocopherol levels were low in all calves fed the basal diet or the basal diet plus selenium. In calves receiving tocopherol supplementation, however, the blood plasma tocopherol levels were high. Calves given no tocopherol supplementation averaged 108 γ of total tocopherol per 100 ml. of plasma, while the calves fed supplementary tocopherol averaged 639 γ per 100 ml., a highly significant difference ($P < 0.01$). The statistical analyses on these disproportionate subclass numbers were based on the method of Snedecor (41) for a $2^2$ factorial design. Serum electrolyte values were normal in all cases, with the exception of magnesium, which fell as the trial progressed. Calves fed the basal diet plus cod-liver oil had serum magnesium levels of 1.5 mg. per 100 ml.; the calves fed the basal diet plus cod-liver oil and selenium had serum mg. levels of 2.1 mg. per 100 ml.; the calves which were fed supplementary tocopherol and selenium had serum mg. levels of 1.6 mg. per 100 ml.; and the calves supplemented with tocopherol had serum mg. levels of 1.4 mg. per 100 ml. These values are low in comparison with normal values of 2.0 to 2.5 mg. of mg. per 100 ml. of serum, as reported by Huffman *et al.* (21) and Blaxter, Rook, and MacDonald (6). No statistically significant differences between the blood magnesium levels on the various diets were obtained. The difference between the initial blood levels of magnesium and the final blood levels was highly significant ($P < 0.01$).

Hemoglobin levels, white cell counts, and differential counts were within accepted normal ranges in all calves and no statistically significant differences were found.

Because of the environmental conditions of dampness and drafts, and because the calves were assembled from several farms, white scours (*Escherichia*

*coli* septicemia) was a problem in these calves. In Table 1 it can be seen that in the calves that completed the treatment period, all of those that did not receive tocopherol supplementation developed Zenker's degeneration of the skeletal muscles and Purkinje fiber degeneration of the heart. Calves 19 (basal diet), 24, 25 (basal diet plus selenium), and 28 (basal diet plus tocopherol) died from *E. coli.* septicemia. Calf 28, which received tocopherol supplementation, died at 30 days of age after a prolonged period of diarrhea. On histologic section, this calf showed a mild Zenker's degeneration of the skeletal muscles. Parrish (35) has pointed out that whenever calves were observed to have a severe diarrhetic condition, concentrations of serum tocopherols decreased.

## DISCUSSION

Under the conditions of this experiment the addition of 1 p.p.m. selenium did not prevent muscular dystrophy in calves which were fed a tocopherol-deficient basal diet supplemented with 30 ml. of cod-liver oil per day.

All of the calves which received the basal diet or the basal diet plus 1 p.p.m. selenium, at the end of the experimental period, had Zenker's degeneration of the skeletal muscles, and degeneration of the Purkinje fibers of the heart. None of the calves which received the tocopherol supplementation had Zenker's degeneration or Purkinje fiber lesions at the conclusion of the experiment. In this experiment 1 p.p.m. selenium, when added to the diet, was not able to replace tocopherol in the prevention of dystrophy. The basal diet, however, contained 0.3 p.p.m. selenium.

The addition of cod-liver oil to the diet produced much more extensive dystrophic lesions than had previously been observed in experimental cases (27). In these experiments the initial plasma tocopherol values were similar; the experiments were conducted during the same time of the year, i.e., late fall and winter, and housing conditions were comparable. The calves showed no clinical signs of muscular dystrophy, with the exception that Calf 17 was recumbent more than the other calves on experiment and held its head slightly to the side during the last week. The latter sign was not observed until seven days after a biopsy of the *semitendinosus* muscle was positive for Zenker's degeneration. During the last 2 wk. on test, Calf 17 was drinking 9.1 liters of reconstituted diet per day and was gaining 0.64 kg. per day. On necropsy this calf had extremely severe Zenker's degeneration, involving almost all of the skeletal muscles of the body. Because calves can be so severely affected with Zenker's degeneration but be eating and growing normally, it is understandable how sudden exercise in the spring aggravates or precipitates Zenker's degeneration. The calves here were in small pens and were not allowed out of them except for a few minutes during cleaning.

Serum magnesium levels were low in all calves in the experiment. The first samples were within the normal range, but subsequent samples showed a marked decline. Magnesium levels in other calves receiving the same diet as these calves but not fed cod-liver oil did not show this decline during a 93-day feeding period

(26). Dehority *et al.* (17) found that the addition of cod-liver oil to the diet of calves caused a decrease in blood serum magnesium and carotenoids.

Blaxter, Rook, and MacDonald (6) and Blaxter and Rook (5) estimated the requirements of calves to be 16 to 18 mg. of magnesium per 100 ml. of diet. Since Blaxter *et al.* (6) did not state the intake of their diet, direct comparisons with the present studies are not possible. On analysis the basal diet fed in the trials reported herein contained 20.2 mg. of magnesium per 100 ml. That this level of magnesium did not prevent low serum magnesium levels may have been because the magnesium was being eliminated as magnesium soaps when combined with undigested fat (36). Duncan, Huffman, and Robinson (18) and Huffman *et al.* (21) showed that magnesium salts were not so well utilized as when the magnesium was present in natural feeds. Two-thirds of the magnesium in the diet fed herein was obtained from magnesium salts. Thomas and Okamoto (43) fed calves whole milk at 10 to 12% of body weight and found a decline in blood magnesium levels at 48 to 131 days of age. When magnesium in amounts equal to that in milk was added as magnesium sulfate, grain, or alfalfa hay, these workers found that the magnesium in grain and hay was utilized better than the magnesium in $MgSO_4$. The differences in utilization were not significant and blood serum magnesium levels declined on all treatments.

All of the calves which developed Zenker's degeneration of the skeletal and tongue muscles also showed degeneration of the Purkinje fibers of the heart. These findings are more fully described in a separate publication outlining the pathology of the experimental cases (22). The report by Moore, Hallman, and Sholl (29), describing the pathology of the calves from the experiments on magnesium deficiency of Duncan, Huffman, and Robinson (18), showed that in ten instances where portions of Purkinje fibers were present in heart sections, six showed extensive degenerative changes of these fibers. These workers also found hyaline degeneration of the skeletal musculature of the calves and it is likely that a concomitant tocopherol deficiency was also present (6). Blaxter, Rook, and MacDonald (6) fortified a magnesium-deficient diet with tocopherol. Calves fed this diet developed hypomagnesemic tetany, but on necropsy showed no degeneration of the Purkinje fibers.

Since the appearance of clinical signs of muscular dystrophy has not been a sufficiently critical criterion for determining the onset of Zenker's degeneration in the experimental calves, more exacting biochemical or other tests must be utilized. Such tests as the analysis for the release of serum glutamic oxalacetic transaminase during muscular dystrophy (42) are being evaluated as suitable criteria.

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# OBSERVATIONS ON THE RUMINAL MICROORGANISMS OF ISOLATED AND INOCULATED CALVES

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**SUMMARY**

A study was made of the microorganisms in the rumen of three calves inoculated with whole rumen contents from a mature cow, and of two calves maintained in the same manner, except that they were not inoculated and were strictly isolated from other ruminants immediately after birth. Data were compared with data previously obtained on normally raised calves. Inoculation of normally raised calves had little effect on the time of establishment of predominant culturable bacteria typical of mature cattle, but had a considerable effect on the age at which ciliate protozoa became established.

When calves were isolated from other ruminants to a far greater degree than is likely under practical farm conditions, the bacterial flora was modified from normal through 17 wk. of age, as indicated by bacterial counts, kinds of predominant bacteria cultured, and an apparent effect of inoculation on the flora of these animals after they reached 17 wk. of age. Some groups of typical ruminal bacteria were present.

Although the number of animals observed was small, it was apparent that the lack of a normal microbiological population did not have any drastic effect on the animals' feed consumption, growth, or health through an age of 17 wk.

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Pounden and Hibbs (22, 23, 24) found, by the use of direct microscopic observations of rumen contents, that the ciliate protozoa and certain morphologically distinct bacteria characteristic of mature cattle were often not readily established in young calves. This was particularly so when calves were somewhat isolated from mature cattle or when high ratios of grain to hay were fed. Inoculation of calves fed predominantly roughage rations with rumen contents from mature cattle hastened the establishment of these microorganisms. Further studies by the Ohio workers indicated that certain aspects of ruminal fermentation as well as the ruminal flora and fauna could be modified by inoculation [see (11) for further references].

They found no advantage of inoculation to weight gains (22), nitrogen retention (10), or other, more direct measures of calf performance (17). Soviet (2) and Israeli (26) workers reported increased growth rate in calves that received ruminal inoculation. Other workers in this country (1, 16, 21) and abroad (18, 25) have been unable to show any advantage of inoculation to calf performance.

The studies indicate that, under most conditions likely to be met, the inoculation of calves would be of little value. However, the studies of the Ohio group indicate that protozoa and certain ruminal bacteria may not be readily established in calves under some conditions. The physiological significance of the bacterial groups observed was unknown. In recent years, considerable progress has been made in the culture and identification of bacteria that appear to be of

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functional significance in the rumen of calves (9, 13) and of mature ruminants (4). The present work was undertaken to determine if the culturable bacterial flora of the rumen of young growing calves could be modified from normal by rather strict isolation from other ruminants, and to determine if inoculation of calves with fresh ruminal contents from a mature animal might accelerate the development of the culturable flora typical of mature animals from that previously observed in calves raised under normal conditions (9). Microscopic observations on ciliate protozoa are included. A preliminary report of this work appeared earlier (6).

## METHODS

The five Jersey calves used, No. 1, 2, 3, 4, and 5, were born on April 26, 6, June 18, May 10 and 20, respectively. Their birth weights were 64, 65, 56, 58, and 59 lb., respectively. Calves No. 1 and 4 were males. The diets fed were the same as previously reported (9). Animals 4 and 5, the isolated group, were removed from the dams as they were born and placed in separate pens, constructed of hog-wire fencing, in a shed with one open side and in which ruminants had not been previously housed. The pens were about 12 ft. apart and were approximately 100 yd. from a closed isolation barn where a maximum of two mature animals were occasionally kept. The closest area where other ruminants were routinely maintained was over 600 yd. away. It was not possible to keep rodents, birds, or other small animals away from these calves.

Precautions taken to limit the transfer of microorganisms from other ruminants to these calves until after 17 wk. of age included the following: Feed and equipment for maintaining the calves and collecting weight data were stored in the isolation area about 1 mo. before the first calf was brought in, and were kept in the area until after calves were over 17 wk. of age. Pasteurized whole milk was brought in daily. The calves were handled and fed by only three persons, who had little contact with the rest of the herd. Overalls, overshoes, and rubber gloves were kept in the area. The gloves were rinsed in 70% alcohol and a solution of disinfectant was walked through whenever the animals were approached.

Calves No. 1, 2 and 3, the inoculated group, were handled the same as in previous studies (9), except that they were drenched with 100 ml. of whole rumen contents within the hour after the inoculum was obtained from a mature cow on an alfalfa hay (79%)–grain ration. The drenching was done immediately after samples of rumen contents were obtained when the calves were 1, 3, and 6 wk. of age.

After the 17-wk. sample was obtained from the youngest isolated calf (No. 5), the isolated calves were inoculated with 100 ml. of protozoa-free rumen fluid. The rumen fluid was prepared as follows: Contents from the alfalfa hay–grain fed animal were filtered through cheese cloth, cooled to 4° C. to slow the motility of microorganisms, and centrifuged at 2,500 r.p.m. for 5 min. in 200-ml. bottles. The supernatant was carefully decanted. The centrifugation and decantation were repeated twice before all entodinia appeared to be absent. The calves were

inoculated immediately after the fluid was prepared and again on two succeeding days with aliquots of the same fluid that was stored under $CO_2$ gas at 4° C.

The rumen contents of these animals were then sampled on the 7th and 14th day after inoculation. They were each inoculated with fresh, whole rumen contents 17 days later and their rumen contents resampled 11 and 21 days later.

Sample collection, observations on protozoa, and cultural methods for counting and isolating bacteria were the same as previously used (9). However, after 20 well isolated colonies had been picked from the RGCA medium roll tubes inoculated with a sample, representative strains of that sample were selected for further study on the bases of oxygen relations, $H_2S$ production, and cellulose digestion, in addition to colony type, morphology, gram reaction, and motility, as previously done.

After representative strains were collected from all samples, they were rechecked for morphology, motility, and gram reaction and tested for the following additional characteristics—final pH and appearance of growth in liquid glucose medium, gas production from glucose, $NH_3$ and indole production, gelatin liquefaction, V.P. test, nitrate reduction, starch hydrolysis, and fermentation of D-xylose, maltose, and glycerol. All of these characteristics were determined by the methods used for gram-negative rods and cocci isolated from anaerobic roll tubes in the previous study (9).

The above characteristics were used to identify presumptively groups of ruminal bacteria previously studied in detail (4, 9). A total of 325 strains was selected for study from the 37 samples to be reported. Twenty-six of these strains failed to grow after storage in the dry ice box and, therefore, could not be completely studied.

## RESULTS AND DISCUSSION

*General observations on the calves.* It should be emphasized that this study was designed to obtain information on the microorganisms of the rumen. The work involved necessitated the use of too small a number of animals to measure accurately animal responses to the treatments.

Table 1 shows data on dry feed consumption and weight gains. None of the data showed significant differences (5% level) between the isolated and inoculated groups. Values for TDN per pound of gain, likewise, did not show significant differences. The data do indicate that the isolated calves compared quite favorably with the inoculated calves in feed consumption and in weight gains.

TABLE 1

Feed consumption, other than milk, and weight gains of the calves through 17 wk. of age

| | Calf No. | Grain | Hay | Weight gains |
|---|---|---|---|---|
| Isolated calves | | | | |
| | 4 | 152 | 335 | 155 |
| | 5 | 110 | 210 | 103 |
| Inoculated calves | | | | |
| | 1 | 120 | 74 | 73 |
| | 2 | 145 | 181 | 112 |
| | 3 | 81 | 153 | 70 |

Calves varied considerably in the ratios of hay to grain consumed. Calf 1 consumed more grain than hay for the entire experiment, while the others consumed more hay than grain after the first 3 to 6 wk. The isolated calves appeared to be more pot-bellied. This appearance might have been a reflection of the greater intake of hay by these animals. No other differences were noted in the general appearance of the two groups.

In general, pH values (Table 2) showed a more neutral value when calves were 1 and 17 wk. of age, but tended to be somewhat acid when calves were 3,

TABLE 2
Data on pH values of rumen content samples of isolated and inoculated calves

| Age (wk.) | Isolated calves | | Inoculated calves | | |
|---|---|---|---|---|---|
| | 4 | 5 | 1 | 2 | 3 |
| 1 | 5.7 | 6.9 | 6.9 | 7.0 | 7.2 |
| 3 | 4.9 | 6.1 | 6.4 | 5.5 | 6.2 |
| 6 | 6.1 | 6.0 | 6.0 | 6.0 | 6.8 |
| 9 | 6.7 | 5.9 | 6.0 | 5.9 | 6.1 |
| 13 | 7.0 | 6.0 | 6.0 | 6.1 | 6.3 |
| 17 | 7.0 | 6.4 | | 6.6 | 6.4 |

6, 9, and 13 wk. of age. The low pH of 5.5 in Calf 2 at 3 wk. of age correlates with a large amount of grain consumed (1.9 and 1.7 lb. per day on the day before and the day of sampling). This was the first calf placed on experiment. Because it was desired to keep the grain consumption at a relatively low figure, the animals were limited to 1 lb. of grain mixture to 6 wk. of age, and 2 lb. thereafter. The low pH of 4.9 for Animal 4 at 3 wk. of age did not correlate with high grain or hay consumption. There appeared to be no significant differences in pH values between the isolated and inoculated calves. However, the data suggested a difference in pH values of rumen contents between the two isolated calves at 9, 13, and 17 wk. of age.

*Protozoa.* As might be expected, the ciliate protozoa did not become established in the isolated calves until they were inoculated with whole rumen contents when they were 24 and 21 wk. of age. When observed 11 days after inoculation, all groups of protozoa (Table 3) were well established in both calves.

TABLE 3
Observations on the occurrence of protozoa in the rumen contents of inoculated calves[a]

| Age | Calf 1 | | | Calf 2 | | | Calf 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Entodinia | Diplodinia | Holotrichs | Entodinia | Diplodinia | Holotrichs | Entodinia | Diplodinia | Holotrichs |
| (wk.) | | | | | | | | | |
| 1 | − | − | − | − | − | − | − | − | − |
| 3 | + | + | − | − | − | − | + | − | − |
| 6 | + | + | + | + | − | − | + | + | + |
| 9 | + | + | + | + | + | + | + | + | + |

[a] The rumen contents of isolated calves contained none of these protozoa until inoculated with whole rumen contents.

It is evident that the protozoa became established in the inoculated calves at a very early age (Table 3). Previous results indicated that none of the protozoa was established in calves raised in the same barn and on the same regime, except that they were not inoculated until some time after 13 wk. of age, at which time they were placed in another barn and had direct contact with mature animals (9).

The results suggest that inoculation of calves greatly accelerates the establishment of protozoa. This is in agreement with results of other workers, indicating that rumen inoculation or close contact with animals harboring the protozoa is necessary before they are established [see (12)]. The fact that protozoa were established in Calf 2 at a later time than in Calves 1 and 3 suggests an effect of the large amount of grain consumed by this animal before and during the time of inoculation and sampling at 3 wk. of age. Others have shown that establishment of ciliate protozoa may be prevented by high grain intake (13, 22).

*Bacterial culture counts.* Table 4 shows the various culture counts of bacteria. There were no significant differences (5% level) between the means of the total anaerobic counts (RGCA medium) when isolated and inoculated calves were compared on the basis of samples taken at a given age. However, when the 6, 9, 13, and 17 wk. anaerobic counts were grouped, there was a significant difference (5% level) between the isolated and inoculated calves. No significant differences in anaerobic counts were found when counts from inoculated or isolated calves were compared on the basis of age. However, the inoculated calves showed a

TABLE 4

Numbers of bacteria obtained by various culture counts from rumen contents of inoculated and isolated calves

| | Isolated calves | | | Inoculated calves | | | |
|---|---|---|---|---|---|---|---|
| Age | 4 | 5 | Mean | 1 | 2 | 3 | Mean |
| *(wk.)* | RGCA medium *(anerobic count, billions/ml)* | | | | | | |
| 1 | 3.9 | 4.0 | 3.9 | 9.8 | 7.2 | 2.1 | 6.4 |
| 3 | 8.0 | 12 | 10 | 5.5 | 28 | 25 | 20 |
| 6 | 28.0 | 18 | 23 | 0.65 | 9.5 | 3.6 | 4.6 |
| 9 | 17.0 | 29 | 23 | 2.3 | 4.5 | 2.5 | 3.1 |
| 13 | 14.0 | 12 | 13 | 1.2 | 1.3 | 1.1 | 1.2 |
| 17 | 3.0 | 15 | 9 | — | 0.88 | 0.27 | 0.57 |
| | Cellulose medium *(MPN cellulolytic bacteria, millions/ml)* | | | | | | |
| 1 | < .02 | < .02 | <0.02 | 16 | 3.3 | < .02 | <6.4 |
| 3 | <0.20 | <0.2 | <0.2 | 22 | < .2 | 13 | <12 |
| 6 | 7.9 | 280 | 144 | 160 | 54 | 540 | 250 |
| 9 | 0.68 | 26 | 13 | 1.7 | 24 | 54 | 27 |
| 13 | 2.2 | 54 | 28 | 110 | 35 | 160 | 100 |
| 17 | 17.0 | 540 | 280 | — | 92 | 92 | 92 |
| | Lactate medium *(millions/ml)* | | | | | | |
| 1 | 50 | 800 | 430 | 130 | 2.5 | 550 | 230 |
| 3 | 1,200 | 1.0 | 600 | 450 | 600 | 1,700 | 920 |
| 6 | 160 | 2,100 | 1,100 | 35 | 130 | 12 | 59 |
| 9 | 140 | 120 | 130 | 170 | 55 | 1.0 | 75 |
| 13 | 80 | 55 | 68 | 32 | 8.5 | 6.5 | 16 |
| 17 | 17 | 200 | 110 | — | 6.0 | 5.5 | 5.8 |

trend toward higher counts at 1 and 3 wk. of age and a lowering through 17 wk. The 13 and 17 wk. samples from these calves were in the normal range for mature animals on similar rations (5, 9).

The trend in anaerobic counts with age in these inoculated calves appears to differ from counts previously obtained on uninoculated calves (9), in which case counts were about the same from 1 through 13 wk. of age and were about double those expected in a mature animal. The failure of the counts to rise at 3 wk. of age in these animals might be explained by their lower consumption of dry feed during that period as compared to the present animals. The fact that the present inoculated animals had anaerobic counts typical of mature animals, and the previous uninoculated calves had higher counts at 13 wk. of age, might be explained by the absence of ciliate protozoa in the latter animals. Guiterrez and Davis (15) suggested that ruminal ciliates might affect the numbers of bacteria in the rumen, because most of them have been shown to feed on bacteria. Also, the protozoa compete with the bacteria for carbohydrate energy sources (20).

Anaerobic counts of the magnitude found in the isolated calves during the 6 to 17 wk. period have not been obtained using the present cultural method, except in younger calves (Table 4), in animals fed mainly grain mixtures (5, and unpublished data on an animal fed a feed-lot, bloat-provoking ration), and animals on lush pasture (unpublished data on animals fed blue grass, mixed clover, or Ladino clover). In the present case, neither the ration nor the age of the animals can explain the counts. It seems possible that the high counts reflect the abnormal flora (see below) that might be less efficient per culturable cell than the normal flora in utilizing substrates of the feed. It is of interest that, though both of these animals tended to have high counts, results given below indicate that they contained substantially different predominant culturable bacterial species.

The cellulolytic bacterial counts appeared to be somewhat lower in the isolated calves than in the inoculated calves at 1 and 3 wk. of age. From 6 wk. of age and on, cellulolytic counts of isolated Calf 5 were similar to counts of inoculated calves, which were mainly in the normal range for mature cattle (9). However, the cellulolytic counts in isolated Calf 4 were low throughout the 17-wk. period. The reason for the very low cellulolytic count in Calf 1 at 9 wk. and Calf 2 at 3 wk. is not known. It may have been due to the very high ratio of grain to hay consumed by these animals on the day of, and the day before, sampling (1.3 lb.: 0.4 lb. and 3.6 lb.: 0.6 lb. for Animals 1 and 2, respectively). However, in other cases of relatively high grain to hay consumption, cellulolytic counts were not depressed. The cellulolytic counts of the inoculated calves of different ages were similar to the counts of uninoculated calves (9), except possibly at 3 wk. of age, where counts of the latter animals appear to be slightly higher.

The counts on lactate-fermenters in inoculated calves were essentially the same as in the calves previously studied (9). The high count in Animal 1 at 9 wk. seems to correlate with the low cellulolytic count and higher grain consumption. The trend in numbers of lactate-fermenters in isolated Calf 4 was similar to the other calves, but no definite trend was apparent in isolated Calf 5.

*Groups of predominant bacteria.* Data on the occurrence of the presumptively identified bacteria are shown in Tables 5 and 6. It should be emphasized that these are only presumptive identifications because of the small number of characteristics determined. Also, the characteristics determined were more distinctive for some groups than for others. The authors are very confident of the identification of the following groups: C1, C2, C5, R3, + R4, *Ruminococcus, Bacteroides succinogenes, Selenomonas, Borrelia,* and *Lachnospira.* A few of the strains were studied in detail and their characteristics are reported elsewhere. These include *Bacteroides ruminicola* strains B888-1 and B932-1 from the 11/4 sample of Calf 4 and B610-1, B742-1, B747-1, and B903-1 from the 13-wk., 9/30, 9/30, and 11/4 samples, respectively, of Animal 5 (8), *Selenomonas* strains B358 and B438 from the 13 wk. samples of Animals 2 and 1, respectively (3), and an unnamed organism, Strain B385, similar morphologically to *Selenomonas* but physiologically similar to *Butyrivibrio,* from Animal 3 at 3 wk. (3). B385 and similar strains were placed in the unknown group (Tables 5 and 6).

In addition, 11 strains of curved motile rods selected from the isolated animals, that appeared to be atypical of the curved motile rod groups previously described, were studied for fermentation acids produced in rumen fluid–glucose medium (8). All of these were found to produce mainly butyric, lactic, and formic acids, with acetic acid being produced or consumed, depending on the strain. This suggested that they were atypical strains of the genus *Butyrivibrio.* They were presumptively placed in this group. It was also found that a few of these strains produced quite large amounts of lactic acid and low amounts of butyric acid. This seems quite plausible for members of the genus, especially since Gill and King (14) showed that the concentrations of butyric and lactic acid produced by a strain of *Butyrivibrio* varied inversely and the amount of each produced depended on the composition of the medium. One presumptive strain of *Eubacterium ruminantium* (4), obtained from the 11/4 sample from Animal 5, was found to conform to this species in major fermentation acids, i.e., butyric, acetic, formic, and lactic acids.

In general, the groups of bacteria cultured from the inoculated calves at different ages (Table 5) are remarkably similar to those previously found in uninoculated calves (9). At 1 and 3 wk. of age, the predominant bacteria in both groups of calves were mainly different from those of mature animals, but some bacterial groups predominant in mature cattle were present. By the time the calves reached 6 wk. of age many groups typical of mature animals were well established, but several groups typical only of calves remained. In the previous study, animals at 9 and 13 wk. of age contained predominant bacteria typical of mature cattle, except that the +R3 group of anaerobic lactobacilli, typical of calves, was still present. In the inoculated calves of the present study, the +R3 and +R4 groups of anaerobic lactobacilli and the C3 group of anaerobic streptococci, typical of calves, were still present at 9 wk., but at 13 and 17 wk. of age only groups typical of mature cattle were present. Other similarities between the predominant bacteria of inoculated calves (Table 5) and the formerly studied uninoculated calves (9) include the following: Groups C1, C5, and R1 were

TABLE 5

Strains of certain presumptively identified groups of bacteria isolated from colonies in RGCA medium inoculated with rumen contents of inoculated calves

| Presumptive group | Per cent of strains | | | | | |
|---|---|---|---|---|---|---|
| | 1 (wk.) | 3 (wk.) | 6 (wk.) | 9 (wk.) | 13 (wk.) | 17 (wk.) |
| C1 | 14 (2)[a] | | | | | |
| C2 | | 4 (1, 3) | | | | |
| C3 | 2 (3) | 4 (3) | 12 (3) | 6 (1, 2) | | |
| C5 | 14 (2, 3) | | | | | |
| R1 | 14 (1, 3) | | | | | |
| R3 | 4 (1, 3) | 7 (1) | | | | |
| + R3 | | | 3 (1) | 8 (1, 2) | | |
| + R4 | | 18 (1, 2, 3) | | 12 (2) | | |
| *Ruminococcus* | | 2 (3) | 12 (1, 3) | 8 (1, 2, 3) | 2 (1) | 8 (2, 3) |
| *Bacteroides succinogenes* | | | 6 (3) | | 8 (1, 3) | 6 (2, 3) |
| *Bacteroides ruminicola* | 2 (1) | 7 (1, 3) | 21 (1, 2, 3) | 2 (1) | 9 (2, 3) | 17 (2, 3) |
| *Selenomonas* | | 18 (2) | | 2 (1) | 16 (1, 2) | 6 (2) |
| *Butyrivibrio* | | | 15 (2) | 8 (3) | 9 (1, 2) | 19 (2, 3) |
| *Borrelia* | | | | | | |
| Miscellaneous | | | | 2[b] (3) | 16[b, c, d] (1, 2, 3) | 8[b, c] (2) |
| Unknown | 51 (1, 2, 3) | 40 (1, 2, 3) | 37 (1, 2, 3) | 54 (1, 2, 3) | 42 (1, 2, 3) | 36 (2, 3) |
| Number of total strains | 51 | 55 | 34 | 52 | 57 | 36 |

[a] Numbers in parentheses refer to calves from which strains were isolated.
[b] *Lachnospira*-like strains.
[c] *Succinimonas*-like strains.
[d] *Eubacterium ruminantium*-like strains.
[e] *Succinivibrio*-like strains.

TABLE 6

Strains of certain presumptively identified groups of bacteria isolated from colonies in RGCA medium inoculated with rumen contents of isolated calves

| Sample | Per cent of strains | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 wk. | 3 wk. | 6 wk. | 9 wk. | 13 wk. | 17 wk. | 9/30 | 10/7 | 11/4 | 11/14 |
| Presumptive group | | | | | | | | | | |
| C1 | 6(5)[a] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C5 | 39(4) | 3(4) | 0 | 0 | 3(4) | 0 | 0 | 0 | 0 | 0 |
| R1 | 13(5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| +R3 | 0 | 0 | 0 | 15(5) | 31(5) | 26(5) | 0 | 0 | 0 | 0 |
| +R4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ruminococcus* | 0 | 0 | 3(5) | 0 | 0 | 0 | 9(4, 5) | 17(4, 5) | 18(4, 5) | 18(4, 5) |
| *Bacteroides succinogenes* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6(5) | 0 |
| *Bacteroides ruminicola* | 0 | 26(5) | 8(4) | 3(5) | 6(4, 5) | 0 | 26(4, 5) | 37(4, 5) | 18(4, 5) | 41(4, 5) |
| *Selenomonas* | 0 | 15(5) | 3(5) | 3(5) | 6(4) | 9(4, 5) | 0 | 0 | 0 | 8(4, 5) |
| *Butyrivibrio* | 0 | 0 | 19(4) | 33(4) | 3(4) | 12(4) | 41(4, 5) | 17(5) | 35(4, 5) | 5(5) |
| *Borrelia* | 0 | 0 | 22(4) | 5(4) | 0 | 3(5) | 0 | 0 | 0 | 0 |
| Miscellaneous | 0 | 0 | 3(5)[b] | 0 | 0 | 3(5)[b] | 3(5)[c] | 0 | 12(4, 5)[d,e,f] | 21(4, 5)[c,e,f] |
| Unknown | 42(4, 5) | 56(4, 5) | 43(4, 5) | 53(4, 5) | 52(4, 5) | 47(4, 5) | 20.5(4, 5) | 29(4, 5) | 12(4, 5) | 8(4) |
| Total strains | 31 | 34 | 37 | 39 | 35 | 34 | 34 | 35 | 34 | 39 |

[a] Numbers in parentheses refer to calves from which strains were isolated.
[b] C4-like strains.
[c] *Lachnospira*-like strains.
[d] +R2-like strains.
[e] *Succinivibrio*-like strains.
[f] *Eubacterium ruminatium*-like strains.

found mainly in week-old calves; the C2 and +R4 groups were found mainly in 3-wk. old calves; the +R3 group was found mainly in 6- and 9-wk.-old calves; the R3 group was found only in 1- and 3-wk.-old calves; the ruminococci and *B. succinogenes* which appear to be the more important cellulolytic bacteria of the rumen, first appeared among the predominant bacteria in calves at 3 and 6 wk. of age, respectively.

Some apparent differences in the groups of predominant bacteria found in the inoculated calves of the present study (Table 5), as compared to the previously studied uninoculated calves (9), included the following: (1) the *Butyrivibrio* group was first found in week-old calves in the previous study and in 6-wk.-old calves in the present study; (2) the reverse was true of the *B. ruminicola* group; (3) the C3 group was found in both younger and older calves of the present study, as compared to the previous study; (4) the +R1 and the R2 groups, found only in week-old calves previously, were not detected among the predominant bacteria in the present calves; (5) *Selenomonas* first appeared at 3 wk. in the present study, but at 6 wk. in the former; (6) *Borrelia* was not found in the present calves but only a few were found in the former calves; (7) the failure to find the miscellaneous groups of Table 5 in the uninoculated calves through 13 wk. of age. The latter was possibly the most significant difference in predominant bacteria of the two groups of calves. However, these bacteria are often only a very small part of the predominant bacteria found in mature cattle (9).

The differences noted in predominant bacteria appear to be small as compared to the similarities. It seems doubtful that many of the differences could be ascribed directly to inoculation. The week-old animals showed a few apparent differences, yet the inoculated animals were not inoculated until after this rumen sample was taken. Some of the later differences might have been due to the lack of protozoa in the uninoculated calves. Also, it should be emphasized that the inoculated calves were studied 1 yr. later than the others and there was no adequate control to determine possible differences due to this. It does seem evident that, contrary to observations on ciliate protozoa, inoculation of normally raised calves that have no direct contact with mature animals except for a few hours after birth has little effect on the establishment of a predominant culturable bacterial flora typical of mature animals.

Of the bacteria found in the inoculated calves and in the previously studied normally raised calves (9), the C2, C3, R3, +R4, and *B. succinogenes* groups were not found in the isolated calves through 17 wk. of age (Table 6). During this period, only one strain of cellulolytic *Ruminococcus* was found. The C1, C5, R1, +R3, *B. ruminicola*, *Selenomonas*, *Butyrivibrio*, *Borrelia*, and C4 groups were found and at ages quite comparable to the normal or inoculated calves.

The finding of large numbers of a single group of unknown organisms in many of the samples from isolated calves through 17 wk. of age (see below) suggests another difference between these calves and the normal or inoculated groups. Unknown strains from samples from the latter calves appeared to include only one or a few strains of several apparently different groups.

Also, differences in characteristics of the strains of *Butyrivibrio* and *B. ruminicola* cultured from the isolated calves and the inoculated calves and

mature cattle suggest a difference in the isolated calves. Of five strains of *Butyrivibrio* selected from Animal 4 before inoculation, none fermented xylan. This organism was not cultured from the other isolated calf during this period. Four of nine strains from the isolated calves after inoculation fermented xylan, and six of nine strains from the inoculated calf group fermented xylan. In an earlier study, 34 out of 48 strains from mature cattle fermented this carbohydrate (7). Seventeen out of 20 of *B. ruminicola* isolated from mature cattle fermented xylose, maltose and xylan and hydrolyzed starch (8). Of eight strains selected from isolated calves before inoculation, only two had these characters. None of the other fermented xylan and only one fermented xylose. After inoculation, nine out of 20 were typical. Fourteen of 18 strains from the inoculated calf group were typical in these characteristics.

These apparent differences, the observations on unknown groups, and comparison of the data in Tables 5 and 6 and the previously collected data on normal calves (9) indicate that, though some predominant groups of bacteria typical of normal and inoculated calves were present, the over-all picture of the flora of the isolated calves was substantially different through 17 wk. of age. This conclusion is supported by the differences shown between isolated and inoculated calves in total anaerobic counts in samples taken during the 6 to 17 wk. period.

A point of particular interest in this study is that, even though some bacterial groups typically among the predominant ruminal bacteria were present in the isolated calves, some of them were unable to become predominant. One strain of cellulolytic *Ruminococcus* was isolated from Animal 5 at 6 wk. of age, demonstrating conclusively that it was present. Yet, no other strains of this bacterium were found until after the animal was inoculated. The strains present in this animal were possibly different from strains present in normal animals in some way that kept them from becoming predominant. It seems equally possible that some unknown organism or organisms that produce materials required for the ruminococci to become predominant were not available to the animal until it was inoculated.

The possibility remains that many of the bacterial groups typically found in calves and mature cattle but not found in the isolated calves were present, and in numbers equivalent to other animals, but could not be detected because of the unusually high numbers of other bacteria found during the 6 to 17 wk. period.

It would be of interest to determine rates of various ruminal reactions, such as cellulose digestion and production of gases and various organic acids, of similar animals in which an abnormal culturable bacterial flora was proved to be present.

The source of the typical ruminal bacteria found in the isolated calves is unknown, but it is possible that some or all of them originated from nonruminant sources. Some bacterial groups typical of the rumen and found in the isolated calves, e.g., *Selenomonas* and *Ruminococcus*, have been found in the gastrointestinal tract of nonruminants [see (4)]; and it seems probable that, if an adequate search were made, others would be found. The calves were not kept from possible contact with rodents, birds, and other small animals. Although

much work remains to be done concerning the possible nonruminant sources, the authors do not believe that many regularly functional rumen bacteria will be found to originate from feed sources.

It can be seen (Table 6) that there were considerable differences in presumptively identified predominant bacteria from the two isolated calves.

Also, there were substantial differences between unknown strains isolated from these two animals. Major groups of unknown bacteria isolated from Calf 4 were as follows: Sixteen of 20 strains from the 3-wk. sample were gram-positive, facultatively anaerobic rods presumed to be lactobacilli because of the low final pH (4.0–4.1) in glucose medium. In the 13-wk. sample, 8 of 17 isolates were gram-negative, anaerobic, starch-hydrolyzing, but otherwise inactive, small spirilla. In other samples from this animal, few unknown strains (1, 6, and 9 wk. samples) or a large variety of unknown groups (17-wk. sample) were found. Major groups of unknown organisms isolated from Calf 5 were as follows: Nine of 17 strains from the 6-wk. sample were gram-positive, anaerobic rods with central swellings and final pH in glucose medium of 4.6-5.2. Five of 19 strains in the 9-wk. sample were motile anaerobic diplococci that were inactive in all other characteristics. Five of 17 strains in the 13-wk. sample were the same as those in the 6-wk. sample and four of the motile diplococci were found in the 17-wk. sample.

These observations on unknown strains plus the data in Table 6 show that from 6 through 13 wk. of age culturable bacteria in Calf 4 consisted mainly of gram-negative bacteria and from 6 through 17 wk. of age in Calf 5 they consisted mainly of gram-positive bacteria.

The observations on predominant bacteria cultured and the apparent differences in ruminal pH values and cultural counts of lactate fermenters, and, particularly, cellulolytic bacteria, indicate that the ruminal flora of the two isolated calves differed considerably. This was true even though the calves were separated in the shed by only 12 ft. and no precautions were taken to limit the transfer of organisms from one calf to the other. The reason for this difference is not apparent. The calves ate similar ratios of hay to grain, made similar weight gains per pound of TDN consumed, and the flora and fauna of both were essentially normal after inoculation (see below).

*The effect of inoculation on the ruminal flora of isolated calves.* Table 7 shows the various cultural counts obtained from the isolated calves after inoculation. The total anerobic count seems to show little effect of inoculation with protozoa-free rumen fluid, except that the count in Animal 5 may have been slightly depressed. After inoculation with whole rumen contents the counts were depressed and about equal to values of the older calves in the inoculated group (Table 4). The effect of inoculation with protozoa-free rumen fluid on the cellulolytic counts apears to be quite drastic. The 10/7 sample from both animals and the 9/30 sample from Animal 4 were higher than the authors have obtained using this method on rumen contents from any other cattle. One might have expected the counts in Animal 4 to increase as they had been lower than normal before inoculation. However, counts in Animal 5 increased greatly, even though they were in

TABLE 7

Bacterial counts of rumen contents from isolated calves before and after inoculation with protozoa-free and whole rumen contents

| | Calf No. | After inoculation with protozoa-free rumen fluid[a] | | After inoculation whole rumen contents[b] | |
|---|---|---|---|---|---|
| Date of sample | | 9/30 | 10/7 | 11/4 | 11/14 |
| | | *(numbers per ml.)* | | | |
| RGCA medium *(anaerobic count, billions)* | 4 | 6.3 | 3.8 | 0.45 | 0.64 |
| | 5 | 11 | 5.2 | 0.50 | 0.36 |
| Cellulose medium *(millions)* | 4 | 70 | 920 | 70 | 160 |
| | 5 | > 1,800 | > 1,800 | 70 | 92 |
| Lactate medium *(millions)* | 4 | 13 | 13 | 4.0 | 6.0 |
| | 5 | 17 | 12 | 1.0 | 4.0 |

[a] Calves were inoculated with protozoa-free rumen fluid on September 21, 22, and 23. The last samples taken before the 9/30 samples were the 17 wk. samples (Tables 4 and 6). These were taken on September 6 and 19 for Calves 4 and 5, respectively.
[b] Calves were inoculated with whole rumen contents on October 24.

the normal range before inoculation. After inoculation with whole rumen contents, the counts were normal.

Results in Table 6 show that the normal balance of predominant groups of bacteria became established after inoculation. Of particular interest was the rapid response of the *Ruminococcus* group in both calves and the *Butyrivibrio* group in Calf 5.

Although no uninoculated, isolated animals of similar age were available, it seems highly likely that most of the changes were due to inoculation. These results are another indication that the flora of these animals was abnormal through 17 week. of age, because it is unlikely that inoculation would have any appreciable effect on animals with a normal flora.

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# EFFECTS OF FEEDING AUREOMYCIN[1] TO LACTATING DAIRY COWS[2]

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**SUMMARY**

Sixty-two pairs (33 Jersey and 29 Holstein pairs) of milking cows were used to compare aureomycin supplementation with a control ration for a 12-wk. period. Treated cows were fed at the rate of 10 mg. per 100 lb. body weight. Seventeen pairs were continued on the experiment for 17 wk. and another eight pairs for 6 mo. The differences between the two groups of cows in milk production and body weight changes were not significant at the 5% level. The cows receiving aureomycin showed no obvious advantage in resistance to mastitis, foot rot, or other bacterial disturbances throughout the study.

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Aureomycin and other antibiotics have been used successfully in the rations of simple-stomached animals for some time. Early reports on the feeding of aureomycin to ruminant animals were somewhat contradictory. Bell *et al.* (3) reported that aureomycin had adverse effects on beef steers and Colby *et al.* (5) showed that it depressed feed consumption and reduced the growth rate of lambs. At the same time, Rusoff (12), Loosli and Wallace (9), and Bartley *et al.* (1) reported that aureomycin fed to young dairy calves promoted growth and was beneficial in helping to reduce scours. A subsequent review by Lassiter (8) in 1955 substantiated the beneficial effects of feeding aureomycin to young dairy calves. Since young calves are not entirely dependent upon rumen microflora, it was suspected that their response to aureomycin was different from that of older ruminating animals. Thus, the use of aureomycin as a feed supplement for mature dairy animals lagged, primarily due to the early reports of the adverse effects when fed to ruminants.

Later studies by Loosli and Warner (10) and Rusoff and Haq (13) showed that the feeding of high levels of aureomycin daily to milking cows produced no harmful or beneficial effects on milk production and feed consumption. Likewise, Bartley *et al.* (2) found that the daily feeding of 300 to 500 mg. of aureomycin per cow had no effect on milk yield, body weight changes, or on the general health and well-being of the animals. Jacobson *et al.* (7) found no advantage in milk or fat production in feeding aureomycin for 15 mo. prior to parturition and during the first lactation.

It is known that the presence of antibiotics in milk will render it useless for cheese production. Reports by Loosli and Warner (10), Rusoff and Haq (13),

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[1] Trademark of the American Cyanamid Company for the antibiotic, chlortetracycline, which was furnished by the American Cyanamid Company, Princeton, New Jersey.

[2] A portion of this work was conducted in cooperation with the Dairy Cattle Research Branch, Beltsville, Maryland.

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and Martin *et al.* (11) give evidence that the feeding of relatively high levels of aureomycin did not affect milk composition or result in measurable amounts of the antibiotic in the milk. Henderson *et al.* (6) reported that no aureomycin was in the blood or milk of animals fed the antibiotic at the level of 0.1 mg. per pound of body weight. Shor *et al.* (14) also found that milk from cows fed aureomycin at the same level did not contain the antibiotic.

Since the feeding of aureomycin to dairy cattle has aroused much interest recently, this study was made to determine the response of lactating dairy animals when fed this antibiotic at recommended low levels.

## EXPERIMENTAL PROCEDURE

Lactating dairy cows of two breeds were paired on the basis of milk production, body weight, age, stage of lactation, and stage of gestation. In addition, the health records which included the prior incidences of foot rot, mastitis, and other bacterial infections were considered in pairing the cows. Sixty-two pairs of cows were included in the study (33 pairs of Jerseys and 29 pairs of Holsteins). The animals were located in four of the University dairy herds.

Aureomycin, in the form of Aurofac 2A which contained 3,600 mg. per pound, was fed daily to one cow of each pair. Sufficient Aurofac 2A was fed daily to furnish aureomycin at the rate of 10 mg. per 100 lb. of body weight. The average body weight of the cows of the same breed in each herd was determined and aureomycin was fed to all cows in the supplemental group on the basis of the average weight of the group. Thus, each cow of the same breed in each herd received the same amount of Aurofac 2A on top of its concentrate mixture. The paired mates in the control group received the same amount of concentrates but were not fed aureomycin at any time. Aureomycin was introduced into the ration gradually over a five-day period. However, a preliminary test at this station showed that milking cows suffered no ill effects when started abruptly on aureomycin feeding at the recommended levels. All cows were barn-fed throughout the greater portion of the experiment. In most cases, the cows were fed mixed hay with either corn or grass silage, all of which was medium to fair in quality. The cows in both groups had access to the same quantity and quality of roughage.

Two-week pre- and post-experimental periods were conducted during which time the paired mates in both groups were managed alike. The rate of aureomycin feeding was gradually increased over a five-day period until the treated cows received 10 mg. per 100 lb. of body weight. The adjustment period was followed by a 12-wk. experimental period, beginning the latter part of December. This season was selected since it is a time when the herds usually experience some difficulty with foot rot. In one herd involving 11 pairs of Holsteins and six pairs of Jerseys the study continued for 17 wk. and in another herd eight pairs of Jerseys were kept on the experiment for 6 mo.

Records were kept of daily milk production and monthly butterfat tests for each cow. Body weights were recorded at the beginning and end of the study. In addition, daily observations of all cows were made to detect incidences of mastitis, foot rot, and digestive or respiratory disturbances.

The daily milk production data were analyzed for statistical significance by an analysis of variance method as outlined by Burt (4).

## RESULTS AND DISCUSSION

No difficulty was encountered in getting the cows to consume the Aurofac 2A when it was placed on top of the concentrate mixture. None of the cows showed ill effects from the treatment.

The average daily milk production for all pairs by weeks is shown in Figure 1. The daily average production of the 29 pairs of Holsteins for the entire period

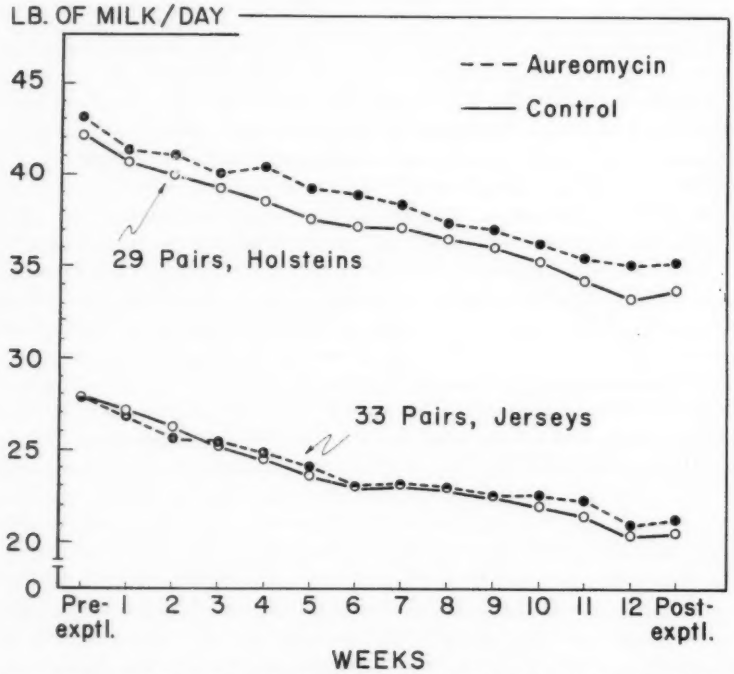


FIG. 1. Average daily milk production of aureomycin-fed and control cows of two breeds.

was 36.9 lb. for the controls and 38.1 lb. for the treated cows. Inspection of Figure 1 reveals that the Holsteins in the supplemented group started on the experiment at a higher level of production and declined at approximately the same rate as the controls. The milk production of the two groups of Jerseys was approximately the same throughout the study. The average daily production was 23.2 lb. for the control Jerseys and 23.4 for the treated Jerseys. The data (especially for Jerseys) in Figure 1 indicate that the supplemented cows were producing slightly more milk than the controls at the end of the 12-wk. period. However, the differences between the two groups were not statistically significant. This was still

true when the data were considered within the two breeds and within the four herds.

When the milk produced was converted to a 4% FCM basis, the two groups were not significantly different. The average daily production of the 4% FCM was 32.7 lb. for the controls and 33.0 lb. for the aureomycin-fed group. These averages again reflect the slightly higher production of the supplemented group which was evident at the beginning of the experiment.

The body weight changes showed no conclusive trend. The average change in weight during the experiment showed that the cows in both groups gained weight. The control group gained an average of 19 lb. per cow, whereas the supplemented cows increased an average of 21 lb. Two reasons may be partially responsible for the increases in body weight. Many of the cows were in the advanced stages of gestation at the end of the experiment and some of the Holsteins were first-lactation heifers that were still growing during the trial.

The health records kept on the two groups of cows in each of the four herds failed to show an advantage for the antibiotic-supplemented group. Mastitis was a problem of concern in only one herd. In this herd, three cows in the control group were treated two or more times for mastitis, whereas five cows in the aureomycin-fed group were treated one or more times for mastitis. In the three other herds combined, only four control cows were treated for mastitis and five of the antibiotic-fed cows required treatment. Throughout the study, four of the control cows were treated for foot rot and six cows in the supplemented group were treated one or more times.

Other noticeable difficulties were limited in both groups of cows. One control cow was treated for metritis and one supplemented cow was treated for another bacterial infection. Two cows in the supplemented group went off-feed for a short period of time. This difficulty was not attributed to the antibiotic, since it occurred after the cows had been receiving the supplement for some time.

In the herd in which the trial was conducted for 17 wk., a Hotis test and a microscopic examination for mastitis organisms were made monthly on every cow. During this time, the milk from each quarter was checked carefully four times, at monthly intervals. The data collected from similar tests before the feeding trial were used in pairing the cows. The first test during the experiment was made after the study had been in progress approximately 30 days. Additional tests were made from January through April. The results of these tests failed to reveal a difference between the two groups of cows. Two cows in each group were positive in one or more quarters. The milk from one or more quarters of eight cows in the control group reacted as though the cow were a suspect for mastitis, and five cows in the supplemented group reacted in this manner. More of the supects in both groups showed up in the last two tests (March and April) than in the first two.

To accumulate data on the effects of prolonged feeding of aureomycin, eight pairs of the higher producing Jerseys in one herd were continued on the experiment for 6 mo. Each of the eight cows in this group received the same amount of aureomycin daily for the entire period. This continuation was of special

interest, since the treated cows shown in Figure 1 appeared to produce at a higher level near the end of the 12-wk. period. The data on these cows are summarized in Table 1. The eight cows in each group produced at approximately the same level. The increase in production during the fifth month corresponds to the

TABLE 1

Average daily milk production by months for eight pairs of Jerseys comparing aureomycin-fed and controls over a 6-mo. period

| Month | Controls | Aureomycin-fed | Difference |
|---|---|---|---|
| | | *(lb.)* | |
| 1 | 38.9 | 38.2 | −.7 |
| 2 | 34.4 | 33.8 | −.6 |
| 3 | 31.7 | 31.6 | −.1 |
| 4 | 31.5 | 32.0 | +.5 |
| 5 | 32.3 | 32.3 | .0 |
| 6 | 29.6 | 28.8 | −.8 |

time that the cows were on spring pasture. Both groups of cows increased in production and declined thereafter at approximately the same rate.

Under the conditions of this experiment, the addition of aureomycin to the dairy ration failed to produce a significant advantage in milk production during a 12-wk. period. Even when cows were continued on the study for as long as six mo., no differences were found between the control and supplemented cows.

It is believed that the disease level of the herd, which presumably is lower in most experimental herds, may influence the response to aureomycin supplementation. The clinical disease challenge was low in the four experimental herds used in this study. Therefore, these results may not be indicative of the responses in herds experiencing higher disease levels.

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# CORRELATION OF MILK PRODUCTION WITH QUALITY AND QUANTITY OF HERBAGE INTAKE UNDER ROTATIONAL AND DAILY STRIP GRAZING

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**SUMMARY**

Chromium oxide and plant pigments have been used as indicators to determine herbage digestibility, fecal output, and digestible and total herbage intake for five cows rotationally and daily strip grazing alfalfa-bromegrass (*Medicago falcata* and *Bromus inermis*) pasture during the grazing season of 1955. Morning and afternoon fecal samples were composited in the manner of three-day running averages to provide these estimates on a daily basis. The relationship of these variables and milk production to time has been discussed and arguments advanced to support representing time as a discontinuous arithmetic series instead of a simple arithmetic series in this and a previous experiment. Consequently, multiple correlation analyses have been recomputed for the two cows used in the previous experiment and are reported with the analyses for the five cows in this experiment. Although there was a marked linearity of decline in lactation with advancing time, daily milk production was also significantly related to variations in herbage digestibility and live weight. These were the main variables related to milk production under strip grazing. Under rotational grazing, digestible and total intake and fecal output were also significantly associated with milk production.

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A study in pasture ecology, designed to compare two grazing management systems, must take cognizance of the interdependence of the pasture and the grazing animal. These are not static entities, but complex biological systems in constant interaction. The ideal in pasture management is maximum efficiency of herbage utilization commensurate with continued productivity of the pasture and adequate nutrition of the grazing animal. The interdependence of pasture and cow is influenced by their mutual independence with regard to time, weather, age, stage of lactation, fertility, etc.

Brundage *et al.* (3) used $Cr_2O_3$ and plant pigments as indicators to estimate quantity and quality of herbage intake by two grazing dairy cows. Combining morning and afternoon fecal samples in the manner of three-day running averages enabled them to make these estimates on a daily basis. They used multiple correlation analysis to study the relationship of milk production to quantity and quality of herbage intake independent of time.

The experiment described in this paper concerns a similar study involving six dairy cows grazing alfalfa-bromegrass (*Medicago falcata* and *Bromus inermis*) pasture under rotational and daily strip grazing systems of pasture management. The objective was to study the relationship of daily milk production to daily herbage digestibility, total and digestible intake, fecal output, and live weight variations within the continuum of time. The correlation analysis was summarized at the annual meeting of the American Dairy Science Association in 1958 (1).

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## EXPERIMENTAL PROCEDURE

This digestibility-intake study was conducted in 1955 to further study the relationship of daily milk production to daily herbage digestibility, total and digestible intake, fecal output, and live weight variation within the continuum of time. The experiment was incorporated as part of a 3-yr. investigation of the efficiency of herbage utilization from alfalfa-bromegrass pasture under rotational and strip grazing systems of management. Details of the over-all design of the 3-yr. trial and results have been presented previously (2).

One-half of a seven-acre alfalfa-bromegrass pasture was subdivided into three equal paddocks and rotationally grazed by four lactating dairy cows. The other half was daily strip grazed by a similar group of four cows. Cows under rotational grazing were not moved from their paddock until those under strip grazing had grazed a comparable area. This restriction of movements between paddocks gave both groups equal opportunity to utilize the available herbage. The pastures were grazed from May 30 through July 26 and from August 23 through September 8. The time spent on any one paddock varied from five to eight days.

Three cows under each management system were employed in the digestibility-intake phase of the experiment. These animals received no supplemental feed while on pasture. They were weighed every other day prior to going to the pasture after the morning milking. Limiting drinking water to an outside tank after weighing reduced the variation of water fill in these data. Milk was weighed at each milking and sampled for butterfat analysis (Babcock) every ten days. These data were used to compute daily production of 4% fat-corrected milk.

Ten grams of $Cr_2O_3$ were administered to each cow via 1-oz. gelatin capsules each morning at 8 A.M. Grab samples of feces were taken from the rectum daily at approximately 7:30 A.M. and 4 P.M. No difficulty was experienced in the morning collection, but occasionally one or more cows were found to be devoid of feces at the afternoon collection. This resulted in a delay of an hour or more until feces could be obtained.

The morning and evening fecal samples were composited into three-day samples in the manner of a running average (3). A total of 73 samples was prepared for each cow and analyzed for $Cr_2O_3$[1] and plant pigments. A Beckman Model B spectrophotometer was employed for the latter analysis. These data were used to compute daily apparent dry matter digestibility, fecal output, digestible intake, and total intake for each cow according to the method of Reid *et al.* (5). Only digestibility and fecal output are independently determined in this procedure. Digestible and total intake are mathematically as well as biologically related to digestibility and fecal output.

One cow under strip grazing was poisoned during the experiment. Data for her have been discarded as being atypical. Her illness did not result from the experiment per se.

[1] The $Cr_2O_3$ analyses were performed by the Wisconsin Alumni Research Foundation, 506 N. Walnut Street, Madison 1, Wisconsin.

## RESULTS AND DISCUSSION

The partial correlations of milk production with digestibility and digestible intake independent of time were computed when milk production was assumed to occur the day before, the same day as, and the day after fecal output. For these and subsequent calculations, the three-day composite fecal samples were assumed to be an estimate of the middle day included in each sample.

In the original experiment (3), and in the preliminary report on these data (1), time was represented as a simple arithmetic series (1, 2, 3, . . . $n$) corresponding to the total days ($n$) on the experimental pastures. It was tacitly assumed that, inasmuch as this representation of time was common for both milk production and the other variables under consideration, partial correlation analysis would give the true relationship between milk production and digestibility or digestible intake independent of time.

Further examination of the data has impugned this assumption, however. The variables under study are not mutually related to time. Lactation and live weight variations are continuous over the grazing season. Variation in quantity and quality of herbage intake are also continuous over the grazing season, corresponding to morphological changes in the pasture with advancing time. At the same time, however, these variations are discontinuous or cyclic in nature, corresponding to the periodic defoliation and regrowth of the pasture throughout the successive grazings during the season.

If the pasture were adequate to maintain the animals continuously throughout the course of the experiment, time would be a continuous function and could be represented by a simple arithmetic series. But, in these experiments, where it became necessary to remove the animals from the experimental pasture to allow regrowth for subsequent grazing, time is more accurately represented as a discontinuous arithmetic series; thus: (1, 2, 3, . . . $a$, $a + b + 1$, $a + b + 2$, . . . $n$) where ($a$) is the number of days grazed before withdrawal from the pasture, ($b$) is the number of days the animals are absent from the pasture, and ($n$) is the total time elapsed during the course of the experiment.

Regarding a discontinuous time series as more realistic than a simple time series for both the original experiment (3) and the one under discussion (1), the correlation analyses have been recomputed for all seven cows. The partial correlations of milk production with digestibility and digestible intake independent of the linear effects of time, when milk production is assumed to occur the day before, the same day as, or the day after fecal output are listed in Table 1.

The 1955 data in Table 1 were transformed to $z$ values and subjected to analysis of variance (6), to test the significance of the differences in partial correlation coefficients obtained. Differences among the two cows under strip grazing were not significant. Differences among the three cows under rotational grazing were highly significant when the variance due to the interaction of cows and measurements (digestibility and digestible intake) was removed from the error variance. Comparison of the means by Duncan's multiple range test (4) showed the partial correlations to be highest (p less than 1%) when milk production was assumed to occur the day after fecal output.

TABLE 1

Partial correlations of digestibility and digestible intake with milk production independent of the linear effects of time, when milk production is assumed to occur the day before, the same day, or the day after fecal output

| Cow | Grazing system | Day before | | Milk production | | | |
|---|---|---|---|---|---|---|---|
| | | | | Same day | | Day after | |
| | | Dig. | Dig. intake | Dig. | Dig. intake | Dig. | Dig. intake |
| | | | | 1954 | | | |
| 209 | Rotational | +.248 | +.213 | +.365[b] | +.172 | +.349[b] | −.018 |
| 210 | Strip | +.171 | +.162 | +.345[b] | +.175 | +.433[b] | +.073 |
| | | | | 1955 | | | |
| 212 | Rotational | +.117 | +.394[b] | +.294[a] | +.350[b] | +.347[b] | +.421[b] |
| 215 | Rotational | +.543[b] | +.430[b] | +.550[b] | +.303[b] | +.610[b] | +.396[b] |
| 227 | Rotational | +.484[b] | +.404[b] | +.589[b] | +.370[b] | +.589[b] | +.434[b] |
| 214 | Strip | +.157 | +.096 | +.297[a] | −.012 | +.321[b] | +.057 |
| 218 | Strip | +.365[b] | +.015 | +.414[b] | +.027 | +.360[b] | +.162 |

[a] Significant at the 5% level.
[b] Significant at the 1% level.

For the more detailed correlation analyses to be discussed, it has been assumed that fecal output preceded milk production by one day. It is necessary to establish this relationship because the variables under study (i.e., digestibility, fecal output, etc.) are determined from the chemical analysis of the series of fecal samples. Hence, the assumption is that digestibility, fecal output, etc. are influencing milk production the day following the appearance of the fecal residues from which these values have been determined. Allowing for the passage of the herbage residues through the posterior portion of the gut, this indicates a considerable delay in time for the expression of these variables in milk production.

The multiple correlation analysis of the data was conducted according to the procedure outlined by Brundage *et al.* (3). First, the simple correlation coefficients of all variables were computed (Table 2). The 1955 data were computed on a within-cow basis for each management system. Inspection of this table reveals negative correlations between time and milk production in all cases. Many of the other simple correlations are impossible to explain biologically. For example, it is difficult to rationalize negative correlations between milk production and digestibility or herbage intake. This supports the contention that these biological functions are confounded with time and must be studied with reference to time.

The other variables were systematically introduced into the linear correlation of time and production and the resulting reduction in error variance for milk production tested for significance. When more than one three-factor correlation gave significant variance reduction, the variables concerned were combined into a four-factor correlation coefficient and tested for significance. This procedure was continued until all the variables significantly related to milk production had been established.

These correlation coefficients are summarized in Table 3. Digestibility was significantly related to milk production in all cases. Under rotational grazing,

TABLE 2

Daily means, standard deviations, and simple correlation coefficients for time, digestibility, fecal output, digestible intake, total intake, live weight, and 4% fat-corrected milk, when milk production and live weight are assumed to occur the day after fecal output. Standard deviations and correlation coefficients for 1955 have been computed on a within-cow basis (two cows under strip grazing and three cows under rotational grazing). The means for 1955 are general means

| Variable | Grazing system | Year[a] | Digestibility (%) | Fecal output (kilo) | Digestible intake (kilo) | Total intake (kilo) | Live weight (lb.) | FCM (lb.) |
|---|---|---|---|---|---|---|---|---|
| Time | Rotational | 1954 | +.514 | +.176 | +.623 | +.539 | +.704 | −.900 |
| | | 1955 | −.058 | +.471 | +.469 | +.494 | +.284 | −.787 |
| | Strip | 1954 | +.673 | −.419 | +.041 | −.100 | +.671 | −.904 |
| | | 1955 | −.602 | +.539 | +.161 | +.333 | +.679 | −.949 |
| Digestibility | Rotational | 1954 | 65.53±3.75 | −.335 | +.605 | +.344 | +.252 | −.332 |
| | | 1955 | 63.21±2.41 | −.358 | +.265 | +.034 | +.045 | +.370 |
| | Strip | 1954 | 67.31±2.05 | −.139 | +.494 | +.321 | +.640 | −.472 |
| | | 1955 | 64.27±2.00 | −.574 | +.114 | −.172 | −.385 | +.662 |
| Fecal Output | Rotational | 1954 | | 3.78±0.64 | +.536 | +.764 | +.348 | −.296 |
| | | 1955 | | 4.94±0.82 | +.801 | +.919 | +.195 | −.362 |
| | Strip | 1954 | | 3.87±0.49 | +.784 | +.889 | −.053 | +.304 |
| | | 1955 | | 4.55±0.61 | +.745 | +.904 | +.517 | −.556 |
| Digestible Intake | Rotational | 1954 | | | 7.25±1.38 | +.955 | +.520 | −.566 |
| | | 1955 | | | 8.48±1.38 | +.972 | +.233 | −.156 |
| | Strip | 1954 | | | 7.97±1.16 | +.981 | +.327 | −.006 |
| | | 1955 | | | 8.16±0.92 | +.959 | +.300 | −.137 |
| Total Intake | Rotational | 1954 | | | | 11.03±1.81 | +.521 | −.538 |
| | | 1955 | | | | 13.42±2.09 | +.230 | −.244 |
| | Strip | 1954 | | | | 11.84±1.57 | +.225 | +.090 |
| | | 1955 | | | | 12.71±1.43 | +.412 | −.325 |
| Live Weight | Rotational | 1954 | | | | | 1222±31 | −.729 |
| | | 1955 | | | | | 1018±20 | −.217 |
| | Strip | 1954 | | | | | 1117±27 | −.690 |
| | | 1955 | | | | | 1023±25 | −.718 |
| FCM | Rotational | 1954 | | | | | | 21.6±3.8 |
| | | 1955 | | | | | | 25.7±3.3 |
| | Strip | 1954 | | | | | | 27.2±5.4 |
| | | 1955 | | | | | | 28.1±5.7 |

[a] 1954— 5% r = .262 1% r = .340
1955—rotational grazing 5% r = .134 1% r = .176
strip grazing 5% r = .165 1% r = .214

TABLE 3

Simple and multiple correlation of milk production with time, digestibility, fecal output, digestible intake, total intake, and live weight, when milk producton and live weight are assumed to occur the day after fecal output. Data for 1955 have been computed on a within-cow basis (two cows under strip grazing and three cows under rotational grazing)

| Variables | Rotational grazing | | Strip grazing | |
|---|---|---|---|---|
| | 1954 | 1955 | 1954 | 1955 |
| Time and FCM | −.900 | −.787 | −.904 | −.949 |
| Time, dig., and FCM | +.912[b] | +.851[b] | +.923[b] | +.956[b] |
| Time, fecal output, and FCM | +.910[a] | +.787 | +.908 | +.950[a] |
| Time, dig. intake, and FCM | +.900 | +.823[b] | +.905 | +.949 |
| Time, total intake, and FCM | +.902 | +.804[b] | +.904 | +.949 |
| Time, live weight, and FCM | +.910[a] | +.787 | +.911 | +.954[b] |
| Time, dig., fecal output, and FCM | +.915 | +.863[b] | +.931 | +.956 |
| Time, dig., dig. intake, and FCM | +.916 | +.863[b] | +.929 | +.956 |
| Time, dig., total intake, and FCM | +.916 | +.863[b] | +.930 | +.956 |
| Time, dig., live weight, and FCM | +.919[a] | +.852 | +.942[b] | +.961[b] |

[a,b] Reduction in error variance for FCM significant at [a] 5% and [b] 1%.

time, digestibility, and live weight gave the highest significant correlation coefficient in 1954, and time, digestibility, and intake (total or digestible) or fecal output in 1955. The combined use of fecal output and intake variables did not significantly improve the correlation coefficients when they were used individually with time and digestibility. This is attributed to the fact that these variables are confounded with each other mathematically as well as biologically. The combination of time, digestibility, and total intake gave the greatest reduction in error variance for milk production.

Under strip grazing, time, digestibility, and live weight gave the highest significant correlation coefficient both years; even though the addition of live weight to time and production alone did not give a significant improvement over the linear correlation.

Multiple regression equations of milk production on the significant variables are presented in Table 4. As anticipated, lactation is negatively related to time, declining as the grazing season progresses. Milk production is also negatively related to live weight variations. This may be a true physiological relationship or it may be illusory, arising from the concurrent decline in production and

TABLE 4

Multiple regression equations of daily 4% fat-corrected milk on time, digestibility, total intake, and live weight

| Year | Number of cows | FCM ($Y$) | Time ($T$) | Digestibility ($D$) | Intake ($I$) | Live weight ($w$) | | Std. error |
|---|---|---|---|---|---|---|---|---|
| | | (lb.) | (days) | (%) | (kilos) | (lb.) | | |
| Strip grazing | | | | | | | | |
| 1954 | 1 | $Y =$ | $-.229\ T$ | $+.910\ D$ | [b] | $-.053\ w$ | +33.57 | ±1.86 |
| 1955 | 2[a] | $Y =$ | $-.142\ T$ | $+.423\ D$ | [b] | $-.033\ w$ | +41.14 | ±1.59 |
| Rotational grazing | | | | | | | | |
| 1954 | 1 | $Y =$ | $-.146\ T$ | $+.156\ D$ | [b] | $-.019\ w$ | +39.87 | ±1.54 |
| 1955 | 3[a] | $Y =$ | $-.092\ T$ | $+.436\ D$ | $+.264\ I$ | [b] | − 1.39 | ±1.70 |

[a]—Computed on a within-cow basis.
[b]—This variable does not significantly enter this equation.

increase in live weight with advancing lactation and pregnancy. Daily milk production is positively associated with herbage digestibility and intake.

Average daily milk production and digestibility for the 1955 experiment are illustrated in Figure 1. These data have been presented previously for the two other cows (3). It is to be expected in a study of this nature, extending

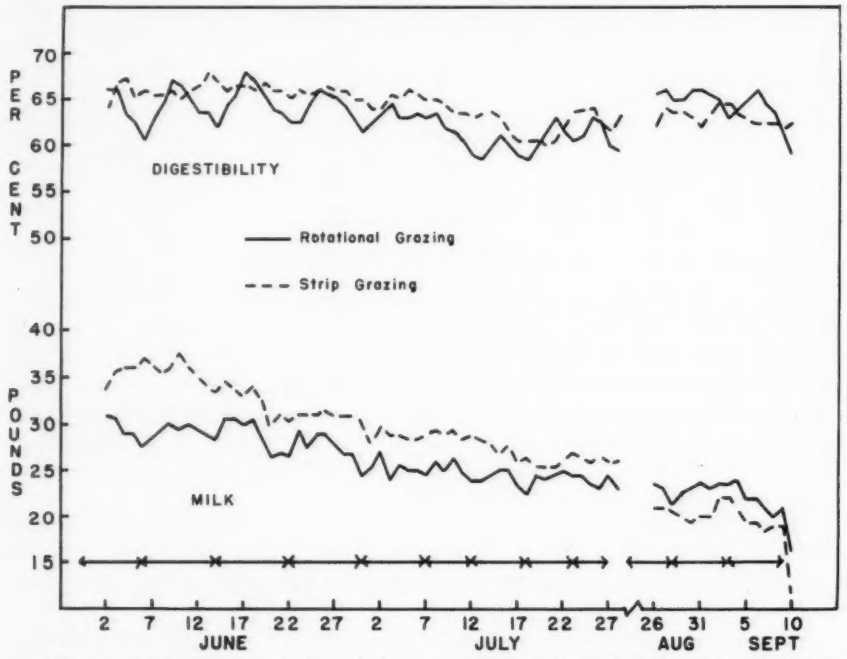


FIG. 1. Average per cent digestibility and pounds fat-corrected milk for cows under rotational grazing (212, 215, 227) and daily strip grazing (214, 218). Solid lines—rotational grazing; broken lines—strip grazing. The lines at the bottom of the graph indicate rotations from paddock to paddock during the experiment.

for several months, that the passage of time will exert a negative influence on daily milk production. Indeed, the negative correlation for time and milk production exceeds −.9 for three cows managed under strip grazing. In spite of this high linear relationship, milk production responded independently to small variations in digestibility at a mean of 65%. This is striking evidence of the dairy cow's sensitivity to small daily variations in digestibility of ingested herbage under the strip grazing system of management. This also refutes the conclusion that variations in digestibility have no effect on milk production under strip grazing, which was based on correlation analyses computed assuming time as a simple arithmetic series (1).

Cows managed under rotational grazing do not exhibit as marked linearity of decline in milk production as those under strip grazing. Production of all

four cows responded significantly to variations in digestibility at a mean of 63.8%. Figure 1 illustrates the cyclic nature of digestibility under rotational grazing. Digestibility rises when the cows enter a new paddock, and then declines as the available herbage is progressively depleted.

It is perhaps redundant to point out that these data are limited in replication and give only a brief and fragmentary view of the dynamic relationship between the pasture sward and the grazing animal. They do appear to be reasonably consistent from year to year. They lend support to the thesis that the lactating dairy cow is sensitive to small variations in quality of ingested herbage, even when utilizing the relatively uniform herbage provided by daily strip grazing. Cows under rotational grazing also responded positively to variations in quantity of daily herbage intake.

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# DEVELOPMENT OF TECHNIQUES FOR EVALUATING GRAZED FORAGE [1]

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**SUMMARY**

Techniques were developed to sample grazed forage with steers, using both esophageal and rumen fistulas. Comparisons between feeds of known composition and samples collected from these same feeds through fistulas indicated that some changes in composition occurred. These changes in composition of certain constituents were influenced by the type of feed and type of fistula. Regression equations for the various changes were calculated. The relative amounts of organic constituents were modified by fistula sampling. The amount of protein and ether extract was the same in the fistula sample as the feed, but significant changes were noted in the amount of crude fiber, NFE, and energy. Fistula samples were highly contaminated with mineral matter. The composition and amount of saliva secreted while consuming a sample of forage was investigated, to explain some of the chemical changes noted in fistula samples. Repeated feed sampling on the same day with the same animal had little effect on the composition of fistula samples. Due to the high variation between feeds, and the generally high correlations between feed and fistula samples, it was felt that the fistula sample represents the best estimate of the foraging animal's diet until adequate comparisons of grazed forage have been made. Fistulated animals were used as sampling agents on pasture. The fistula sample could be separated botanically, between grasses and broad-leaved plants.

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The importance of grazing land in American agriculture is well established. It is desirable to obtain suitable methods by which the nutritive value of various range and pasture forages can be determined. A number of systems have been developed to determine the quality and quantity of the grazing animal's diet, but much of the work was based on unproved assumptions or other means of estimating the animal's grazing habits. The biases that occurred and consequent errors might not have been measurable if grazing animals were unable to select their diet. Several outstanding investigations have shown that animals selectively graze (3, 4, 5, 7, 8, 9). Many have felt that the only practical means of pasture and range forage evaluation is through the use of the animal as the sampling agent. The indicator method has gained wide acceptability in recent years as a method for determining both nutritive value and intake of grazed forage; however, a representative sample of the animal's diet can not be obtained with the use of the indicator method alone. Accurate forage sampling using the animal was accomplished by Torell (11), with the establishment of a successful esophageal fistula in a sheep. Recently (2) a method for establishing a cannula in esophageal-fistulated sheep for the enhancement of forage collection has been developed. Work by Bath (1) indicated that only ash appeared to be higher in fistula-collected samples when compared to feed samples. No changes were re-

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ported in other chemical constituents. Additional work (12) indicated that mineral contamination is very high in forage collected from esophageal-fistulated sheep.

The purpose of this study was to (1) develop suitable biological sampling techniques with steers, to further investigate the intake and nutritive value of grazed forages, and (2) make chemical comparisons between feeds of known composition and samples collected from these same feeds through fistulas.

## EXPERIMENTAL PROCEDURE

Rumen fistulas were established in three yearling steers. The operation was similar to the two-phase method reported by Schalk and Amadon (10). Rumen fistulas were closed with a simple plug constructed with two pieces of masonite lined with foam rubber. One piece was placed inside the rumen and the other on the outside. Two or three smaller pieces of masonite, approximately the same size as the fistula, were used as fillers between the two larger plates and held in place with a 5/16- × 4-in. carriage bolt and wing nut. This system allowed the plug to be closed tightly enough to prevent most leakage, but not tightly enough to cause necrosis.

The following procedure was used to collect samples from rumen-fistulated animals: The animal was first restrained and the rumen plug removed; next, the entire contents from the rumen and reticulum were removed and placed in a suitable container. The sides of the rumen were cleaned by downward strokes with the hand. This step was essential to lessen contamination of the collected sample. After the rumen was evacuated, the animal was allowed to sample the forage for 30 to 45 min. At this time the animal was again restrained, the ingested sample removed, and the former rumen contents replaced in the rumen.

Esophageal-fistula cannulas were established in four steers. The operation was similar to that described by Cook *et al.* (2) for sheep. After the animal was anesthetized with a general anesthesia, an incision was made through the skin and subcutaneous fascia in the upper one-half of the cervical region, and then between the left margin of the sterno-hyoid muscle and the right margin of the sterno-cephalicus muscle. Passing a stomach tube into the rumen prior to the operation aided in locating the esophagus. An incision somewhat shorter than the length of the flanged end of the cannula was made in the esophagus and the cannula was then inserted into the esophageal lumen. The tissue was closed tightly around the cannula by suturing the anterior and posterior ends of the incision in the esophagus with No. 2 catgut. Next, the subcutaneous tissue was sutured tightly, which aided in securing the cannula. The incision through the skin was then closed with Vetafil[3] and dusted with sulfanilamide. Following the operation the animals were maintained on pelleted feed to facilitate healing.

The first cannula used in an esophageal fistula consisted of a modified "T" made from plastic pipe. The inside diameter was 1½ in. and the flanged end was

[3] Vetafil is a heavy-type suture (0.40-mm. diameter) manufactured by Fort Dodge Laboratories, Fort Dodge, Iowa.

approximately 5 in. long. The cannula was closed with a rubber stopper held in place with a water hose clamp. Cannulas used in a later operation were of a larger size. These were modified from a "T" made of a plastic material[4] with an inside diameter of 2 in. and a flanged end 5½ in. long. This material was ground to a thinness of approximately ⅛ in. to reduce weight and add flexibility. Closure of the cannula was accomplished with a screw cap. A two-piece esophageal fistula plug (11) was used to close fistulas upon removal of the cannula from certain animals.

Esophageal fistula collections were made simply by removing the screw cap, or plug, depending on the type, and placing a collection sack around the neck. The collection sack, with a capacity of approximately 3 gal., was constructed of rubberized canvas and held in place with a strap around the top of the neck. Time required for collection of samples from esophageal-fistulated animals was similar to rumen-fistulated animals.

Comparisons between feeds of a known composition and fistula samples of these feeds were made. Alfalfa-concentrate pellets, alfalfa hay, and grass hay were tested on both types of fistulas. A limited number of grass and alfalfa samples was fed in the succulent stages. The sample of feed to be tested was divided into two parts with one-half used as the control sample and the other half fed to the animal and collected through the fistula.

In a later study, alfalfa hay was used to determine both the effect of fistula sampling and the effect of consecutive sampling within a comparatively short time period on the chemical composition of the fistula-collected sample. A two by four factorial design with three replications was employed for this study. Three animals with each type of fistula were compared on four consecutive sampling periods with a time interval of 1.5 hr. between sampling periods. Rumen contents were withheld from the rumen-fistulated animals for the entire period. Animals were not allowed access to water during the entire sampling period.

In a related study, the amount of salivary contamination in rumen-fistula samples was determined with several levels of grass hay. Salivary secretion was determined by feeding a known quantity of hay which was recovered from the rumen along with the saliva secreted. The difference between the initial weight and the final weight was recorded as the amount of saliva secreted while consuming the sample. The experimental design for this study was a three by three factorial with three replications. Three different initial weights of hay were used with three animals. During this study, limited numbers of saliva samples were collected for chemical analysis. A 400-ml. beaker was placed under the cardia in the anterior portion of the rumen. Several types of feed were presented to the animal, but he was not allowed to sample them in an attempt to stimulate the secretion of saliva comparable to that secreted when actually consuming forage.

[4] Vulcathene "T's" (No. T34) were purchased from American Vulcathene, Division of the Nolge Co., Inc., Rochester, New York.

## RESULTS AND DISCUSSION

Rumen-fistula techniques used in this study were satisfactory. However, esophageal fistulas were not entirely successful. The first cannula, 1.5 in. inside diameter, was successfully established but proved too small for a bolus to fall through. The cannula was removed and a plug was fitted to the fistula. The animal was maintained on pasture for 3 mo. under these conditions. On a grass hay diet, the animal developed trouble in swallowing. Several times the esophagus became compacted with food and the fistula opening gradually became larger. The animal could not be maintained under these conditions. The larger cannula was established in the next two operations. The cannula was accidentally removed from one animal shortly after the operation. Failure to devise an adequate plug resulted in death 2 mo. later. The operation appeared successful on the second animal in this series, the wound healed, and little scar tissue developed. The animal was used successfully for 75 days; however, the anterior lip of the flanged end finally protruded through the esophagus and skin, and the animal was sacrificed. A later attempt to establish a more modified 2-in. "T" also failed.

Sixty-two comparisons of fistula and feed samples indicate that some changes occur with fistula sampling by steers. The change in amounts of certain constituents through fistula sampling is influenced by the type of feed and type of fistula (Table 1). The regression equations for the various changes were calculated with $X$, the independent variable, representing the fistula sample, and $Y$, the dependent variable, representing the usual feed sample. Through the application of these regression equations to the appropriate feeds and types of fistulas, it will be possible to predict the chemical composition of the foraging animal's diet. When the regression equation is not significant, but significant changes occurred by fistula sampling, the composition can be determined simply by adding the recorded change.

The amounts of certain organic constituents were modified by fistula sampling. In all cases, the amounts of ether extract and protein were the same in the fistula sample as the feed, but significant changes were noted in the amount of crude fiber, nitrogen-free extract (NFE), and energy.

The amount of crude fiber in fistula samples of grass hay and alfalfa-concentrate pellets increased, but NFE decreased. It is possible that soluble portions of NFE might be leached from the feed by the saliva and lost in sampling. If this effect occurred, it would explain the results obtained, although these soluble carbohydrates should be collected with the esophageal fistula. Esophageal fistula samples usually contained more NFE than rumen fistula samples, but this effect was statistically significant only when alfalfa hay was fed. The reason for the increase in crude fiber in fistula samples, using alfalfa-concentrate pellets and grass hay, is not clear. The approximate analysis adds up to 100%; therefore, a change in the value of one constituent should cause relative changes in the values of the others. Thus, indirectly, the decrease in NFE may account for some of the increase of crude fiber in fistula samples. NFE and crude fiber accounted for more than 60% of the composition of roughages. Over-all NFE decreased significantly less with alfalfa hay than it did on other feeds involved in this

TABLE 1

A comparison of feed and fistula samples of the same feed

| Constituent | Type of feed | Type of fistula | Feed-fistula sample[a] | Regression equation | Correlation value |
|---|---|---|---|---|---|
| Crude fiber | Grass hay, alfalfa-concentrate pellets | Both | $-2.27\% \pm 0.68^{**}$ | $\hat{Y}= 2.02+0.867X^{**}$ | .929** |
| | Alfalfa hay | Esophageal | $+4.06\% \pm 1.77^{**}$ | $\hat{Y}= 7.94+0.900X^{**}$ | .621** |
| NFE | Grass hay, alfalfa-concentrate pellets | Both | $+4.19\% \pm 0.91^{**}$ | $\hat{Y}=21.04+0.590X^{**}$ | .615** |
| | Alfalfa hay | Rumen | $+1.53\% \pm 1.36\%$ | $\hat{Y}=17.99+0.614X^{**}$ | .622* |
| Energy (kcal/gram) | Alfalfa hay | Rumen | $+0.059 \pm 0.036^{**}$ | NS | NS |
| | All types of feed | Esophageal | $+0.077 \pm 0.028^{**}$ | $\hat{Y}=1.509X-2.132^{**}$ | .851** |
| Energy (ash-free) | All types of feed | Both | $-0.086 \pm 0.022^{**}$ | $\hat{Y}=2.298+0.515X^{**}$ | .556** |
| Ash | Grass hay, alfalfa-concentrate pellets, succulent alfalfa | Both | $-2.02\% \pm 0.38^{**}$ | $\hat{Y}=2.22 +6.28X^{**}$ | .630** |
| | Fibrous alfalfa hay | Both | $-3.57\% \pm 0.43^{**}$ | NS | NS |
| Phosphorus | All types of feed | Rumen | $-0.18\% \pm 0.03^{**}$ | $\hat{Y}=0.853X-0.110^{**}$ | .851** |
| | All types of feed | Esophageal | $-0.10\% + 0.02^{**}$ | $\hat{Y}=0.867X-0.054^{**}$ | .970** |

* = Significant at $P < .05$.
** = Significant at $P < .01$.
NS = Not significant.
[a] Difference is absolute and is determined by subtracting composition of fistula sample from feed sample, i.e., a change of −2.27% indicates fistula sample contained 2.27% more of the constituent than did the feed sample. Confidence limits are at the 5% level.
$X$ = Fistula sample. $Y$ = Feed sample.

study; in fact, NFE did not decrease on esophageal-fistula samples of this feed. The majority of the alfalfa used in this study was low in NFE because soluble constituents were leached by rain. Less NFE was available at the time of collection which, in turn, resulted in less difference between the feed and the fistula sample. The decrease in crude fiber in esophageal-fistula samples may be caused by incomplete collection. The more fibrous parts of the bolus may have continued down the esophagus while the less fibrous and smaller portions were collected. When fistulated cattle were fed coarse alfalfa hay, the esophageal-fistula samples were 5% higher in protein than the rumen-fistula samples. Since the protein is primarily in the leaves, these data would support the above hypothesis. This difference did not approach significance because of the large variation associated with this coarse feed. More data are needed to test this hypothesis.

Energy decreased in all cases in fistula samples, although it was not significant with rumen-fistula samples of grass hay or alfalfa-concentrate pellets. This decrease was small and apparently due to ash contamination. When energy was calculated on an ash-free basis, the fistula samples were significantly higher in all cases than the feed. If the fistula samples were corrected for the caloric content of the saliva, there probably would be no change of energy when calculated on an ash-free basis, and on a dry weight basis the decrease due to the dilution of organic matter with ash would be even greater. The caloric content of the saliva was not determined.

The most striking changes in chemical composition of fistula samples occurred in the mineral matter. Ash increased highly significantly in all cases on fistula samples. Closer examination of the data indicated that ash increased significantly more on coarse, fibrous alfalfa than it did on collections made with succulent alfalfa, grass hay, and alfalfa-concentrate pellets. Phosphorus also increased significantly ($P < .01$) on all fistula samples; however, the increase was significantly greater on rumen-fistula than on esophageal-fistula collections. Calcium increased slightly in fistula samples, but not significantly.

The effect of hay consumption on salivary secretion (Table 2) indicated that for the first pound of hay consumed, there were 4.5 lb. of saliva secreted and

TABLE 2
Effect of hay consumption on salivary secretion

| | | | |
|---|---|---|---|
| Pounds of hay consumed | 1.00 | 2.00 | 3.00 |
| Pounds of sample collected | 5.59 | 10.10 | 14.75 |
| Pounds of saliva secreted | 4.49 | 8.10 | 11.75 |

for every pound thereafter, up to 3 lb., an additional 3.6 lb. of saliva were secreted. Other roughages may behave differently in their effect on salivary secretion.

Analysis of saliva (Table 3) indicated that the dry matter is mainly ash. Calcium was recorded in only trace amounts. McDougall (6) obtained similar values for the same constituents in sheep's saliva. With the assumption that all tests were involved with approximately 2 lb. of feed, an animal would secrete 8.1

TABLE 3
Average composition of four samples of bovine saliva

| Dry matter | Organic matter | Total ash | P | | Na | | K | |
|---|---|---|---|---|---|---|---|---|
| | | | in saliva | in ash | in saliva | in ash | in saliva | in ash |
| (%) | | | | | | | | |
| 1.06 | 0.21 | 0.85 | 0.03 | 3.61 | 0.33 | 39.52 | 0.03 | 4.08 |

lb. of saliva, which would account for the increase in ash, phosphorus, and calcium contamination of the fistula sample.

Those fistula samples of coarse type feeds containing more ash may have resulted from greater salivary secretion. Although quantitative data were not available for evaluating the effect of different roughages on salivary secretion, it appears that both the amount and kind of roughage influence the secretion rate. Greater phosphorus contamination of the rumen-fistula sample, when compared to the esophageal-fistula sample, may be due to contamination from the rumen wall.

Repeated fistula sampling had little effect on the composition of the fistula samples. The amount of calcium was significantly different from the fourth period when compared to Period One. No other changes were observed with any of the constituents.

The correlations between the composition of the feed and the fistula sample were high and statistically highly significant for most constituents. Since a variation exists between types of feed, with a high correlation between the composition of feed and fistula samples, the fistula sample probably represents the best estimate of the composition of grazed forage until adequate comparisons of grazed forage, or forage similar to grazed, have been made. It is doubtful if regression equations calculated from hay and fistula samples of hay could correctly apply to succulent pastures.

The collection techniques were successfully applied to pasture studies. Both rumen-fistulated and esophageal-fistulated animals were used as sampling agents. Botanical analyses of the collected samples on irrigated pasture indicated that grass could easily be distinguished from broad-leaved plants. No attempt was made to distinguish clovers from other broad-leaved plants.

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# TECHNICAL NOTE

## OBSERVATIONS ON THE ACCURACY OF THE GRADUATIONS OF BABCOCK MILK TEST BOTTLES

According to the laws of New York State, the maximum error in the total graduation, or in any part of the graduation of a Babcock test bottle, must not exceed the volume of the smallest graduation. All bottles used for commercial and official testing in the state are checked to insure that they meet this requirement.

For use in research, we wished to select bottles having errors much less than the legal tolerance. In order to select such bottles, we constructed a calibrating syringe. Mercury was forced into the necks of the inverted bottles by a plunger whose movement was measured by means of a micrometer graduated to read directly in hundredths of a millimeter. Micrometer readings were recorded at 2% intervals on the graduations. Great care was observed to control reflections in the meniscus, and to avoid errors of parallax. The true values of the graduations were calculated from the movement and diameter of the plunger.

The reproducibility of measurements with this instrument was tested by asking 11 different people to make measurements on the same test bottle. Because some made two sets of observations, 18 sets are available for the comparison shown in Table 1.

From these measurements made by individuals with no previous experience, it is evident that significant measurements can be made under test conditions to 0.01%. The mean values indicate an amazing accuracy for this particular bottle, which was picked at random from stock.

Twenty-four milk test bottles (Kimble No. 1000) were taken from the supply on hand and all were tested by the same individual. The results of these measurements are shown in Table 2. Most of the bottles showed errors of increasing magnitude of the same sign, either plus or minus, along the scale. This suggests that the major source of error in calibration is in the initial measurement of the distance between the 0 and 8% marks, and not in irregularities in the diameter of the neck.

TABLE 1

A comparison of 18 sets of measurements made by 11 people on the same test bottle

| % Marked | Measured value | | | | Standard deviation |
|---|---|---|---|---|---|
| | Mean | Max. | Min. | Spread | |
| 2.0 | 1.9990 | 2.007 | 1.974 | .033 | .008 |
| 4.0 | 4.0007 | 4.013 | 3.985 | .028 | .009 |
| 6.0 | 5.9976 | 6.012 | 5.980 | .032 | .009 |
| 8.0 | 7.9827 | 8.002 | 7.962 | .040 | .010 |

TABLE 2

Errors found in the graduation of 24 milk test bottles

| % Marked | Measured value | | | Standard deviation from marked value |
|---|---|---|---|---|
| | Mean | Max. | Min. | |
| 2.0 | 1.9997 | 2.015 | 1.986 | .0075 |
| 4.0 | 3.9993 | 4.030 | 3.977 | .0135 |
| 6.0 | 5.9924 | 6.033 | 5.959 | .0182 |
| 8.0 | 7.9874 | 8.040 | 7.946 | .0255 |

It must be recognized that 24 bottles constitute an exceedingly small sample of those in use. Nevertheless, the data indicate that Babcock bottles can be graduated quite accurately, and that the accuracy of Babcock testing is not limited by the accuracy of calibration of the bottles.

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# INTERPRETIVE SUMMARIES OF PAPERS

## AUTOXIDATION OF MILK LIPIDS. I. IDENTIFICATION OF VOLATILE MONOCARBONYL COMPOUNDS FROM AUTOXIDIZED MILK FAT

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The reaction of atmospheric oxygen with milk lipids (autoxidation) causes development of oxidized flavors in many dairy products. The dairy industry has been cognizant of this problem for over a half-century, yet a complete understanding of the chemical reactions involved and means for preventing oxidized flavors are still forthcoming. It is known that when fat oxidizes, carbonyl compounds are produced which are major contributors to the oxidized flavor defect. The chemical identity of these compounds is not complete. Such information would be helpful in at least two ways. First, it would be useful for developing an objective chemical test for quality control purposes. Most chemical tests heretofore used are termed indirect, that is, they do not measure the amount of oxidized flavor compounds but measure some other chemical property indicative of the oxidation reaction. These indirect tests have not given good correlations with sensory tests and it is believed that a measurement of the flavor compounds would be more satisfactory. Secondly, chemical identity of the carbonyl compounds of oxidized milk lipids would give us a better understanding of the effects of environmental conditions on the types of flavor compounds produced and the mechanisms of oxidation reactions. This information may reveal better methods for inhibiting this objectionable reaction in dairy products.

The purpose of this investigation was to identify the carbonyl compounds resulting from the autoxidation of milk fat and to determine their approximate concentrations in the fat. Several samples of milk fat were allowed to oxidize to different stages, the volatile carbonyl compounds were isolated, and 18 of these compounds were identified. Some occurred at relatively high concentrations, whereas others were found in trace amounts. The proportionate amount of each of the compounds comprising the mixture undoubtedly is critical in determining the character of the oxidized flavor. Some of the compounds in oxidized milk fat are identical to those in oxidized milk phospholipid systems, but there are also differences in some of the compounds. The fact that some of the compounds reported in oxidized phospholipid systems were not in milk fat, coupled with the importance of the proportionate concentration of each compound in the mixture on flavor character, may help to explain the reported differences in oxidized flavors for fluid milk and milk fat systems.

In fluid milk, the phospholipid fraction is concentrated on the fat globule surface and is exposed to oxygen. Furthermore, the phospholipid fraction contains a high concentration of unsaturated fatty acids. These acids are believed

to be the initial sites for oxidation and it has been assumed, therefore, that the oxidized flavor of fluid milk arises from the phospholipid fraction. In the case of milk fat, a large portion of the phospholipids is lost during preparation of the fat and that remaining in the fat may be distributed throughout the system. Therefore, phospholipid oxidation may be of secondary importance during the oxidation of milk fat. This reasoning has been used by many investigators to explain the difference in the character of oxidized flavors in fluid milk and in milk fat. This paper supports this viewpoint.

## CHEMICAL COMPOSITION OF MILK [1]

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Changes in composition influence the stability of milk during processing or result in defects in cheese, ice cream, evaporated milk, or other dairy products. Many factors are known to influence milk composition, such as breed, stage of lactation, environment, and feeding and management practices. However, little information is available on the extent of these compositional changes in bulk milk supplies; nor is it possible to predict how much variation might occur in a milk supply during the year.

Milk samples were received biweekly from six major milk-producing areas of California. These areas exemplify differences in feed and management practices as well as in breed. Data from 258 milk samples collected during a 21-mo. period were analyzed for 23 components. In addition to total solids, fat, and solids-not-fat, protein fractions and mineral constituents also were determined.

The differences in composition of the milk from the different areas are summarized in tabular form. Statistical significance of compositional differences of milks from different areas and by season also are given. The area differences reflect variations primarily due to breed and are significant for most constituents; exceptions—soluble organic phosphorus, citric acid, and magnesium. Most constituents also show seasonal changes, with a few exceptions. Usually, they are at their lowest levels in May, June, and July and at their highest levels in November, December, and January.

Five constituents—total soluble phosphorus, soluble inorganic phosphorus, soluble organic phosphorus, citric acid, and magnesium—show greater compositional variations due to seasonal changes than due to area differences. These constituents may be responsible for the processing difficulties or defective dairy products that result from seasonal changes in milk composition.

[1] This study was supported in part by funds from the California Dairy Industry Advisory Board.

# COMPARATIVE ACCURACY OF THE PLASTIC BEAD METHOD OF GOLDING AND THE LACTOMETER METHOD OF WATSON FOR ROUTINE DETERMINATION OF SOLIDS-NOT-FAT IN MILK

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A rapid and practical solids-not-fat (SNF) test for cow's milk has four major uses. These are: (a) for purposes of selective breeding; (b) pricing of milk on its total composition; (c) rapid determination of solids in certain manufacturing processes, and (d) screening samples of milk for possible water adulteration.

The lactometer method, now in use over 50 yr., has become the most common method for determining the density of milk. Formulae for converting density measurements to per cent SNF are used.

Though lactometers are of acceptable accuracy for most purposes, they have not gained widespread field use because of their fragility, sensitivity to small changes in temperature, quantity of sample required for testing, and the time required to test a large volume of samples.

In 1957, N. S. Golding described a method for determing SNF, using plastic density beads. Polymethylstyrene (CYMAC 400), the plastic used, reaches constant density when annealed, is practically the same as water in coefficient of thermal expansion between 66 and 76° F., and is completely insoluble in water, milk, and detergents. Ten beads, each 5/16 in. in diameter and varying in density 1 mg. through a specific gravity range of 1.025–1.034, are placed in 4-oz. screw-cap sample jars. Milk is sampled directly into these jars. After tempering at 104° F. for 5 min. the milk samples are thoroughly mixed and cooled. At 68° F. the number of plastic beads on the bottom of the jar are counted.

The purpose of this study was twofold, namely: (a) to develop an average conversion formula based on milk samples from many cows and (b) to compare the relative accuracy of the plastic beads and the large lactometer of Watson's, using the gravimetric assay for reference values.

The conversion formula for plastic beads is: per cent SNF $= 9.13 - 0.279\,B + 0.307\%\,F$ where $B$ is the number of plastic beads sinking at 68° F. and per cent $F$ is the percentage fat in the milk. This formula was calculated using results from 1,873 milk samples from Guernsey, Holstein, and Jersey cows in 53 herds.

In a second experiment, using 300 milk samples from six breeds, the plastic bead test for per cent SNF was compared with the lactometer. As compared with the per cent SNF determined from gravimetric assay, two samples out of every three were within 0.17% SNF for the lactometer and 0.18% SNF for the plastic beads. The plastic bead test has the same relative accuracy as the lactometer and each is sufficiently accurate for the routine testing of milk of individual cows for per cent SNF. The plastic bead test is simpler and more rapid. Approximately 100 determinations can be made per hour by DHIA supervisors.

# AN IMPROVED PROCEDURE FOR THE DETERMINATION OF MILK PROTEINS BY DYE BINDING

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The Kjeldahl procedure for determining protein is accurate, but it is time-consuming. As a result, simpler and more rapid procedures have been sought. Breeders of dairy cattle are eager for such a test because they recognize the nutritional qualities of milk proteins and would like to have a more effective means of making the consumers aware of milk's most valuable nutrient, the proteins. By selective breeding many visualize increasing milk protein. Udy first developed the Orange G dye method as a means for selecting wheat varieties based upon their protein content.

The cheese industry has also recognized the need for a rapid protein method, since the yield of cheese depends upon the protein content of the milk. Some believe that the pricing of milk should be based upon a formula which includes the protein as well as the fat content.

The dye-binding method depends upon the fact that Orange G in acid solution forms an insoluble complex with milk proteins. Within certain limits of dye and protein concentration, the dye removed from solution is proportional to the quantity of protein present. The procedure is rapid and simple. An automatic syringe is used to deliver 1.500 ± .003 ml. of milk into 25.00 ml. of the dye solution buffered at a pH of 2.0. This mixture should stand for at least 30 min. and may be stored for as long as 2 wk. at room temperature in a tightly stoppered bottle. The insoluble complex is then removed by filtration and the amount of dye remaining is determined colorimetrically.

The present work describes a means for increasing the sensitivity of the colorimeter measurements of the dye not bound. The dye-binding procedure then has the same precision as the macro-Kjeldahl method, although it must be standardized by means of the Kjeldahl method. The standard curve is prepared by plotting the colorimeter readings of a series of samples against their known protein content. A special flow-through cuvette having a short light path is used. Otherwise, a known dilution of the filtrate must be made before the amount of dye remaining can be measured. The colorimeter is set at the midpoint of its most sensitive range with a standard dye solution containing 0.500 mg. of Orange G per milliliter. Most milk samples give readings close to this value.

The amount of dye bound per unit weight of protein designated as the dye-binding capacity (DBC) varies from a value of 0.25 for whey proteins to 0.00 for nonprotein nitrogen. In spite of this variation, the mixed proteins of

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milk give a remarkably constant value for the DBC. Variations in the contents of either butterfat or lactose are without effect on the dye binding of the protein.

This improved procedure was statistically compared with the macro-Kjeldahl method on 345 milk milk samples from six breeds. The correlation was .98. Two-thirds of the samples run by dye binding were within 0.08% protein of the Kjeldahl values. There were no significant breed differences. Duplicate dye-binding determinations are unnecessary, since only one determination out of 20 is in error more than 0.06% protein, as compared with the average of duplicate readings.

This method, as accurate as macro-Kjeldahl, can be done as rapidly as the Babcock test for milk butterfat. Since the protein dye complex is stable in the reagent solution in tightly stoppered bottles for at least 2 wk. at room temperature, shipment of samples to central laboratories for final determination is practical.

## STUDIES ON MILK POWDERS. IV. THE FOAM AND SEDIMENT FRACTIONS OF RECONSTITUTED WHOLE MILK

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Reconstituted whole milk usually contains insoluble material. Some of this insoluble material may be observed as a foam or scum at the surface of the milk and as a sediment which deposits upon low-speed centrifugation of the milk. The scum coats the side of the container and gives the milk a displeasing appearance.

The present work was undertaken in the hope that more precise information concerning the composition of these materials would suggest changes in the conditions of manufacture of whole milk powder, which would prevent their formation in reconstituted milk.

Sizeable quantities of scum and sediment were isolated from reconstituted whole milk. The fractions were extracted with ether and various analyses were made on the ether-soluble and the ether-insoluble portions of each fraction.

Melting point and iodine value determinations on the ether-extractable portions of the foam and sediment indicated that the fat of the sediment contained a higher proportion of unsaturated fatty acids than did the fat of the foam. This was confirmed by the results of the fatty acid determinations.

Electrophoretic analysis of the ether-insoluble portions of the foam and sediment showed that the major protein constituent of the sediment was beta-lactoglobulin, and that of the foam was casein.

# SOME BIOCHEMICAL CONSTITUENTS IN SERUM, CEREBROSPINAL FLUID, AND AQUEOUS HUMOR OF VITAMIN A DEFICIENT HOLSTEIN CALVES

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While vitamin A is known to be involved in several relatively specific functions in most animals, the first change observed in experimentally produced vitamin A deficiency of several species, calf, chick, rabbit, and pig, is an elevation in the spinal fluid pressure. Since the causes(s) of this increased pressure is not known, some biochemical constituents in spinal fluid of vitamin A deficient calves were determined so as to gain possible insight into the cause of the increased pressure. Aqueous humor, the watery fluid filling the anterior chamber of the eye, and blood serum were also included, since aqueous humor is formed of the majority of biochemical constituents occurring in fluid and aqueous humor.

Sixteen male Holstein calves, partially depleted of their vitamin A reserves, were each fed daily for a 16-wk. period one of four carotene intakes, the lower two chosen to give marked increases in spinal fluid pressure, the next slight increase, and the highest no elevation. Just prior to or upon the termination of the 16-wk. carotene feeding period, the expected response in spinal fluid pressure was observed. The pressure within the eye, intraocular pressure, was not affected by carotene intake. As would be anticipated, both blood and liver concentrations of vitamin A were directly associated with carotene intake. No other biochemical constituents of blood serum exhibited consistent trends with increasing carotene intake, but in spinal fluid potassium decreased slightly with increase in carotene intake. In contrast to serum and spinal fluid, the biochemical constituents determined in aqueous humor showed change with an increase in carotene intake. These changes were also accompanied by greater osmotic pressure of those calves fed the lower three carotene intakes.

This experiment did not provide information as to causes of the increased spinal fluid pressure in vitamin A deficient calves. However, the observed changes in spinal fluid pressure probably can not be attributed to the transfer of water from serum to spinal fluid, which would be anticipated, for example, if a change in the osmotic pressure of these fluids had occurred. Thus, the hypothesis of Millen and Dickson, 1957, that increased spinal fluid pressure is due to an overproduction and/or an obstruction at the sites of absorption, resulting in greater volume of fluid within the relatively fixed space of the central nervous system, is supported by the results of this experiment.

Why changes in concentrations of some of the constituents of aqueous humor, as well as increased osmotic pressure, were accompanied by no increase in pressure within the eye, require further experimentation.

## NUTRITIONAL MUSCULAR DYSTROPHY IN CALVES. II. ADDITION OF SELENIUM AND TOCOPHEROL TO A BASAL, DYSTROPHOGENIC DIET CONTAINING COD-LIVER OIL

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White muscle disease, muscular dystrophy, causes severe losses of dairy and beef calves at times in certain areas. Alpha-tocopherol (vitamin E) therapy has been effective sometimes in curing the disease, which occurs under practical livestock production, but at other times the vitamin has been ineffective. Recently, selenium has prevented a similar disease in young lambs, but its effectiveness for calves had not been investigated.

Nutritional muscular dystrophy was produced in young dairy calves by feeding them a semi-purified milk diet containing cod-liver oil, but low in tocopherol. Calves on this diet remained healthy when they received a daily supplement of 200 mg. of alpha-tocopherol acetate. Muscle dystrophy was not prevented by 1 p.p.m. of selenium. Since lambs respond to such a dose, it is not known whether the basal diet, which contained 0.3 p.p.m. of selenium, already contained enough so that an additional amount could not show a response, whether the dose was too small, or whether a specie difference is involved.

Degeneration of the Purkinje fibers of the heart was uniformly observed, along with changes in the skeletal muscle and tongue muscle. Death of calves from heart failure, which is often seen on farms but was not observed in the experimental calves because they were sacrificed too soon and not subjected to stress, can easily be explained by the Purkinje fiber degeneration.

Blood plasma magnesium levels declined throughout the experiment in all calves and was not related to supplements fed. No evidence of magnesium deficiency was seen.

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## OBSERVATIONS ON THE RUMINAL MICROORGANISMS OF ISOLATED AND INOCULATED CALVES

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Studies at the Ohio station have indicated that under some conditions, such as when separated from mature cattle shortly after birth, calves may be slow

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in developing certain ruminal bacteria and protozoa typical of adult cattle. It was found that the oral administration of cud material or ruminal contents from adult cattle resulted in the more rapid establishment of these microorganisms in the calf rumen. It was suggested that inoculation of calves fed high roughage rations might have a favorable effect on the calf.

Studies have indicated that some physiological differences are apparent in calves that contain or do not contain these indicator organisms. However, in more direct measures of calf performance, such as weight gains and feed efficiency, the Ohio workers found no differences.

Data on calf performance as affected by inoculation have been collected by many other workers and conclusions have varied, although most workers in this country and in the United Kingdom found no favorable effect. However, in most of these studies clear-cut conclusions as to whether inoculation speeded the development of the typical ruminal flora or whether satisfactory calf performance could be obtained without the rapid development of the typical ruminal flora could not be made.

Also, when the bacteria were studied, they were observed only under the microscope and their physiological significance in the rumen was not known.

In recent years, considerable progress has been made in the culture and identification of bacteria that are of functional significance in the rumen. The present work was undertaken to determine if the culturable bacterial flora of the rumen of young calves could be modified from normal by rather strict isolation from other ruminants, and to determine if inoculation of calves with ruminal contents from a mature animal might accelerate the development of the flora typical of mature animals.

The numbers and kinds of predominant culturable bacteria in the rumen of three calves raised under normal conditions but inoculated with whole rumen contents were compared with similar data on two calves managed in the same manner, except that they were not inoculated and were isolated from other ruminants to a far greater degree than is likely under practical farm conditions. Data were compared with those previously obtained on normally raised calves.

Inoculation of normally raised calves had little effect on the establishment of predominant bacteria typical of mature cattle but, as indicated by others, greatly decreased the time necessary to establish the ciliate protozoa.

The results conclusively demonstrated that, by strict isolation of calves from other ruminants, the bacterial flora can be altered from normal. The predominant species in calves up to 17 wk. of age were mainly different from those normally predominating.

Although the number of animals was small, the isolated calves containing the abnormal flora appeared to be as healthy, consumed as much feed, and made as good weight gains as the other animals.

# EFFECTS OF FEEDING AUREOMYCIN[1] TO LACTATING COWS[2]

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Previous work has shown that the feeding of aureomycin to ruminants helped combat certain infectious diseases and when low levels were fed to lactating cows none of the antibiotic was found in the blood or milk from these animals. In November, 1957, the Food and Drug Administration released the feeding of aureomycin to dairy cows if fed at the recommended level of 0.1 mg. aureomycin daily per pound of body weight. Subsequent reports indicating higher milk production from supplemented cows stimulated much interest among dairymen, and this study was made to gain more information as to the response of lactating animals when fed aureomycin at recommended levels.

Sixty-two pairs of lactating cows (33 Jersey and 29 Holstein pairs), located in four of the University dairy herds, were used in the study. Aureomycin was fed daily for 12 wk. during the winter months. Sufficient Aurofac 2A (3,600 mg. aureomycin per pound) was fed daily to furnish aureomycin at the rate of 0.1 mg. per pound of body weight. Aureomycin was introduced into the ration gradually and fed to each cow on the basis of the average body weight of each breed in each herd.

The average daily production of 4% FCM was 33.0 lb. for the aureomycin-fed cows and 32.7 lb. for the controls. The supplemented cows started on the experiment at a higher level of production and declined at approximately the same rate as the controls. Seven control and ten aureomycin-fed cows were treated one or more times for mastitis, whereas four controls and six cows in the aureomycin group were treated one or more times for foot rot.

No harmful effects were observed from feeding aureomycin to dairy cows at the rate of 0.1 mg. per pound of body weight. In this experiment, the addition of aureomycin to the dairy ration failed to produce an advantage in milk production during a 12-wk. period. Likewise, there was no significant difference between aureomycin-fed and control cows in body weight changes or in the resistance to mastitis, foot rot, or other bacterial infections. Even when cows were continued on the study for 6 mo., no differences were noted.

[1] Trademark of the American Cyanamid Company for the antibiotic, chlortetracycline, which was furnished by the American Cyanamid Company, Princeton, New Jersey.

[2] A portion of this work was conducted in cooperation with the Dairy Cattle Research Branch, Beltsville, Maryland.

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# CORRELATION OF MILK PRODUCTION WITH QUALITY AND QUANTITY OF HERBAGE INTAKE UNDER ROTATIONAL AND DAILY STRIP GRAZING

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Unlike other forages, which are harvested and hand-fed to dairy cattle, pasture herbage is selected and consumed by the grazing animal without human intervention. This intimate relationship between pasture and animal has long precluded direct measurement of grazing intake. The discovery that fecal concentrations of plant pigments could be used as indices to their concentration in the consumed plants permitted indirect digestibility estimates of herbage freely consumed by the grazing cow. The quantity of pasture intake could then be estimated from herbage digestibility and total fecal output, the latter being measured directly by total collection or indirectly through the use of an inert marker, chromium oxide.

The relationship between cow and pasture is not static, but is in a constant state of flux. The combined plant pigment–chromium oxide techniques were used in these investigations to examine the day-to-day variations in quality and quantity of pasture intake under rotational and strip grazing. Daily fecal samples were combined into consecutive three-day samples, dropping one day and adding one day with each succeeding sample, to provide daily estimates of intake while retaining the advantages of composite samples.

Under rotational grazing, herbage digestibility was closely associated with rotations between paddocks. Digestibility rapidly increased when a new paddock was entered, and then declined steadily as the paddock was grazed down. Pasture intake was more erratic, however, and did not show the clearly defined cyclic pattern of digestibility.

Both digestibility and intake appeared to be less variable under strip grazing, neither exhibiting the range in values or the regularity of change found under rotational grazing. By limiting selection to one day's allowance of pasture, the strip grazing system forced the animals to select a more uniform diet. That this was not at the expense of quality of intake is shown by the higher average herbage digestibility under strip grazing: 67.3 and 64.3% vs. 65.5 and 63.2% under rotational grazing.

The relationship of milk production to daily quality and quantity of herbage intake was studied through multiple correlation and regression analyses. Milk production was inversely related to time, representing the normal decline with advancing lactation. Milk production was also inversely related to live weight changes, corresponding to live weight increases with advancing lactation. Production was directly associated with herbage digestibility, even under the relatively uniform diet imposed by strip grazing. Although production under strip grazing was related to time, live weight, and digestibility only, it was also directly related to pasture intake under rotational grazing.

# DEVELOPMENT OF TECHNIQUES FOR EVALUATING GRAZED FORAGE

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It has long been desirable to obtain a suitable method by which the nutritive value of various range and pasture forages could be determined as grazed by the animal. Early workers were hampered in forage research because they could not devise a practical means to accurately measure consumption under grazing conditions. Recent developments using the animal to sample the forage offer the first major advances in evaluating grazed forage.

It was the purpose of this study to develop suitable biological sampling techniques with steers that would measure the nutritive value of grazed forage and also determine the composition of freshly consumed feed as collected through different types of fistulas.

Rumen and esophageal fistulas were used. In esophageal-fistulated animals forage was collected by placing a suitable container under the open fistula while the animal was consuming feed. Collections were made from rumen-fistulated animals by removing all the rumen contents and allowing the animal to consume the feed. The fresh sample was then removed, and the rumen contents replaced. When not in use, fistulas were closed by various types of plugs.

In this study rumen fistulas were more successful than esophageal fistulas. Although esophageal fistulas would appear to be best for this type of work, further refinements will be needed before they will become as useful as rumen fistulas in evaluating forage.

The relative amounts of organic constituents were modified by fistula sampling. No changes were noted in the amounts of ether extract and protein. Fistula samples contained more crude fiber and less nitrogen-free extract (NFE), when compared to the chemical composition of the same feed samples before sampling. Energy was lower in fistula-collected samples. Apparently, the more soluble portions of NFE were leached from the feed by the addition of saliva and lost in sampling. This loss of NFE would tend to concentrate the crude fiber, which would explain the higher content of crude fiber in the fistula sample.

Ash and phosphorus were higher in all fistula samples. Measuring the amount and composition of saliva excreted while consuming the various feeds accounted for the increase both in ash and in phosphorus in the fistula samples.

# SYMPOSIUM

## IMPROVING DAIRY CATTLE BY BREEDING. I. CURRENT STATUS AND OUTLOOK[1]

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For brevity, I will speak in terms of improving production, although the dairyman's ideal involves also efficiency of feed use, type, longevity, etc.—these often being combined in complicated ways. High producing ability is always more desirable than low producing ability. Other things being equal, the higher producing cow will make her owner more profit (or at least will cause him less loss) than the low producing one, no matter what may be the general level of profitability or unprofitability of dairying as an enterprise compared with alternative enterprises.

### PRESENT RATES OF PROGRESS

Genetic improvement can approach 1% per year if we concentrate entirely on production, cull the lowest producers as fast as we can

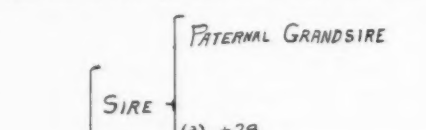


FIG. 1. Expected results of mass selection.

spare them, and use in natural service only the sons of the highest producing cows. The argument, somewhat oversimplified, runs about as follows and is illustrated numerically in Figure 1.

Progress ($\Delta$) per generation, as a result of mass selection, is the product of three factors: $\Delta =$ (accuracy) (genic variability) (intensity). If any one of these three is zero, progress will be zero, no matter how high the other two may be.

*Accuracy* is the correlation between an animal's real transmitting ability and what we think that ability is. For butterfat it is about .4 to .5 for cows with only a first lactation, and rises to around .55 to .65 for cows with three lactations. For progeny-tested bulls, it may be anywhere from lower than .3, where the daughters were few and all in one herd and nearly contemporary, to upwards of .7, where the daughters are many and are either scattered through many herds or corrections for herd differences were made almost perfectly. Progeny tests are rarely as accurate as .8 although, theoretically, they could approach 1.0 under ideal circumstances.

*Genic variability* (the additively genetic standard deviation is the technical genetic term) can scarcely be changed at all by the breeder. Within the pure lines of classical genetics, it is zero. Selection within such pure lines produces no results. But breeds of dairy cattle contain much genetic variation and are far from being pure lines as the geneticists use that term. The genic variation in lactation butterfat yields is about 30 to 40 lb. I will use 35 in the numerical example, as corresponding to an observed standard deviation of 80 lb. and a heritability of 1/5.

*Intensity* is maximum when selection is strictly on the item under consideration, with no exceptions and no attention to anything else. Under those circumstances, and if the distribution is what statisticians call normal, the figure for intensity depends entirely on how large a fraction must be saved. Some of the values it takes are:

| Fraction saved | Intensity |
|---|---|
| .80 | .35 |
| .70 | .50 |
| .60 | .64 |
| .20 | 1.40 |
| .10 | 1.76 |
| .01 | 2.67 |
| .001 | 3.37 |

The first three rows include the possibilities for culling cows. The next two show roughly what might be done in selecting the dams of bulls, if the bulls are to be used in natural service. The last two lines are getting near the limits

[1] Presented in a Symposium at the American Dairy Science Association's Meeting at Urbana, Illinois, June 17, 1959. Journal Paper No. J-3780 of the Iowa Agricultural Experiment Station. Project No. 1053.

of what can be done in selecting bulls if artificial breeding is used. The actual intensity will be less than these figures, perhaps far less, for several reasons, such as: (1) paying attention to other things in the selections and cullings, (2) selling some of the good ones to hold and please customers, (3) not finding some of the potential good ones because they were not tested, etc.

Figure 1 shows, as a numerical example, what we would expect if in each generation we culled the 30% of the cows which have the lowest records and saved sons only from (a) the highest 10% of the cows, (b) the highest 1% of the cows, or (c) the highest 1/10% of the cows. The figures in the grandam's place in the pedigrees are the pounds of fat in breeding value we would expect such selection to have made those grandams to average above the generation in which they were born.

After the first generation, the expected progress with each additional generation is ¼ (the total improvement in the two grandams). With 4 or 5 yr. to the cattle generation, and starting with a herd averaging 400 lb., this yields a rate of improvement as follows:

| | Per generation | Per year | Per cent per year |
|---|---|---|---|
| (a) | 9 | 2 | 0.5 |
| (b) | 12½ | 2.8 | 0.7 |
| (c) | 15½ | 3.5 | 0.9 |

In this example, it was assumed that attention was concentrated wholly on production. We are likely to fall far short of that. On the other hand, no use was made of sib tests, progeny tests, or any pedigree information, except to choose bulls according to the production of their dams. We will do better than that, but doing so will require that we slacken the intensity of individual selection assumed here, so the change is not all pure gain.

Robertson and Rendel in 1950 (Journal of Genetics, 50: 1-8 and 21-31), using the same argument but a slightly higher heritability and with a bit more optimism about the intensity of culling possible, concluded that milk production could be improved about 1% per year. This is a convenient figure to remember. I don't think it can be wrong by a factor of as much as two, but one must remember that they also were computing the maximum rate possible biologically.

Robertson and Rendel went on to ask how much this could be raised by the optimum use of progeny tests and artificial insemination. They computed that the rate thus could be raised from 1.0 to 1.69% per year, if as many as 2,000 cows were being tested in the unit. Most of these 2,000 would be used for progeny-testing young bulls, but the very best cows would be bred to those bulls already proved best. Out of these matings would come the next crop of young bulls to be tested.

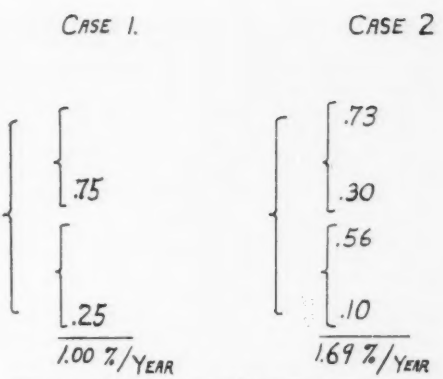


FIG. 2. Maximum possible percentage improvement per year.

Case 1. Natural service and only the cows are selected.

Case 2. Optimum use of A.I., of progeny and sib testing, and of selecting cows: 2,000 cows available in the unit, for testing young bulls and for producing future sires.

Later, they concluded that in a still larger unit the rate could be raised to 2% per year if 10,000 cows could be used for this testing and for producing sire candidates. Figure 2 shows the change in the rate of improvement and the shifting of its sources in their two cases.

The figures for the grandsires in Case 2 show the familiar fact that much can be accomplished by the optimum use of progeny-testing and sib-testing. But the lower figures for the grandams show that the grandsire figures are not all net gain. If, for instance, one-fifth of the young bulls tested are dead or for other reasons not available at five or six, when we first learn their proof, and from among the available ones we use only the one-fourth with the highest proof, then in Case 2 we must start testing five times as many bulls as we need to save for use in Case 1. This decreases considerably the intensity of the selection we can practice for paternal grandams. And if we do any selecting on maternal grandsires, as when we cull a cow because her sire had a poor progeny test (i.e., because her paternal sisters were poor) instead of considering only her own record, we automatically must weaken the intensity of culling on her own record.

The effects of dominance and epistasis are included here with the effects of variations in environment, although they might behave differently enough to deserve separate consideration in a population where linebreeding or other forms of inbreeding are general and intense.

The ultimate limits to which genetic improvement can carry a breed seem utterly unpredictable from any knowledge we have now or seem likely to obtain. The rates of improve-

ment, on the other hand, are as predictable as the accuracy of our knowledge of heritability (in the narrow sense) and of the intensity of selection will permit. The rates of improvement possible are unlikely to change much within as short a time as four or five generations. Hence, the unpredictability of the ultimate limits does not matter in practice. For instance, if my breed averages 450 lb. now under average HIR conditions and I can improve it now at a rate of 4 lb. per year, it is of no practical concern to me that I can not know now whether the ultimate limit on my breed, with the genes it contains now, is 800 lb. or 1,200 lb. If I did know that, I would do nothing differently now although, of course, it would be pleasant to have my curiosity satisfied. The rate of possible change may be expected to vary as the population does, but a reappraisal of that every four or five cattle-generations is likely to be often enough for the practical purposes of knowing when to make any major changes in actual breeding plans. I need not worry in 1959 about what I should do in 2059, although perhaps I should think a little about what to do in 1980, and certainly I should be much concerned to do the best I can in 1960 and even in 1965!

The actual trends over the last 30 or 40 yr., in most countries where dairying is a major enterprise, have been an increase of approximately 2 lb. of fat per year. These trends may be only partly caused by genetic improvement. The environment probably has been improving also, although no wholly satisfactory method for measuring separately the genetic and the environmental parts of these actual trends has yet been devised. Hence, I see no way to be sure whether we are now actually improving our cattle genetically only 1/10th as fast as we might, or perhaps have been doing as much as 50% of the maximum possible.

Here it is necessary to clarify what is really meant when we try to separate a trend into the parts caused by genetic and by environmental improvement. First, the real increase in our knowledge of feeding and other management, and in our ability to control illness and parasites, does not necessarily mean that the environment to which the individual cow is subjected has, therefore, actually improved. Possibly, the benefits of better nutrition, sanitation, etc., may have been partly or wholly cancelled—so far as the individual cow is concerned, although certainly not for her owner—by keeping the cows in larger herds under more crowded conditions with great competition from each other, etc. Second, the fact that the average cow eats more today than she did 20 yr. ago does not prove that her increased production today is caused by our feeding her more. For that, we need to know why she eats more today. If dairymen do take our advice and feed according to production, they build into the data a strong positive correlation between the individual cow's intrinsic producing ability and the amount of feed she receives. If the data then show that the cows who ate the most produced the most, this does not of itself tell us whether they produced the most because they ate the most or whether they got a chance to eat the most because they volunteered to produce the most. This comes close to arguing about which came first, the chicken or the egg! It is imaginable that the amount of feed which a cow can transform into milk has been much increased genetically but that efficiency of feed conversion has not really changed. Or it is imaginable that feed efficiency also may have been improved, although verbally our emphasis on selection has been primarily on amount produced and little, if at all, on any other measure of individual efficiency available to the breeder. We simply do not have the data necessary to unravel that, if, indeed, the attempt to do so would really make any sense.

## NEW KNOWLEDGE NEEDED FOR INCREASING THE RATE OF IMPROVEMENT

1. To make selection more accurate, we need sounder and simpler selection indexes. That requires four kinds of knowledge:

(a) The phenotypic correlations between all traits which are to be considered.

(b) The genetic correlations between all traits.

(c) The heritability of each trait.

(d) The economic importance of each trait.

Already we know much about (a) and quite a little about (c), although more precision in our estimates, especially of (c), would be welcome. About (b), we have only begun to learn. The present methods of estimating genetic correlations have distressingly large sampling errors.

Economic values (d) need more scrutiny, especially in those cases where the economic value of the character changes rapidly as the character is improved. For example, raising extremely low test is likely to be worth while, but each additional increment in test is likely to be worth less than the preceding one. However, one can not afford to follow economic values closely when those are changing erratically. The average generation interval in dairy cattle is something more than 4 yr. One would like to select today in accordance with the economic values which will prevail ten to 20 yr. from now. To do that with complete success would require prophetic ability of a high order; still, it must be done as best we can. The breeder's main task in this respect is to decide which price and other economic variations are only temporary and which are long-time trends. What, for instance, ought we to do now about selecting for solids-not-fat? Von Krosigk, in a still unpublished study, has estimated that in 19 yr. of selecting solely to in-

crease the percentage of protein in the milk, the protein would increase by .28 percentage points, the solids-not-fat by .37, and the fat by .48. If, on the other hand, one selected directly and wholly for total solids, in the same time protein would increase by .25, solids-not-fat by .34, and fat by .64. The latter would be preferable if fat will still be a high-priced product on the market 19 yr. from now, but the former would be preferable if by then the price emphasis will be mainly on protein.

Even when we have these four kinds of knowledge, many problems on how to use them will remain. Information on different characters becomes available at different times. This justifies using preliminary, or screening, tests for early culling of animals which are below certain levels, in order not to waste time or money in maintaining animals until we can learn how good they are in those of their traits which develop late. Still, one must always be careful not to do much of this early culling. Otherwise, he will have no freedom left to do anything about the characters which unfold later.

2. We need to know how to measure separately the effects of epistasis and of overdominance. If the former is important, we need to follow the linebreeding road, making outcrosses only rarely and forming new lines around the most successful of those. If overdominance is important, we make our new lines out of mediocre crosses between lines which all cross well on some other line on which we intend to cross our new lines for producing commercial dairy cattle. To some extent, these two roads are mutually incompatible. The linebreeding road is intended to lead by trial and error through an uncharted wilderness of gene interaction (nicking) to the ultimate goal of pure stocks as good as or better than any crosses which could be produced from them. The overdominance road, if followed successfully, would lead to a goal where the pure seedstocks may be mediocre or less in their own ability, but where their crosses are individually superior to any crosses we can now make or to any pure seedstock whichever could be made. If either or both of those roads are untraversable, knowing that now would be worth much.

3. If overdominance or other causes of heterosis are important enough, the rotation crossbreeding of commercial dairy cattle may be profitable. Now that artificial insemination is widespread, and most studs carry bulls of all dairy breeds, the individual dairyman could follow a regular rotation crossbreeding scheme much more easily than was possible when he used natural service and needed bulls of different breeds for different ones among his cows. So long as high grades are not eligible for registry no matter how good they are, the only considerations, other than the breeder's esthetic feelings which determine whether he should crossbreed, are the facts about how much he will probably gain by the heterosis.

4. A separate question, although not an unrelated one, is whether the overdominance is enough to make it worth while for breeders of seedstock to select their animals explicitly for their ability to cross well. Plant breeders generally call such a proposal reciprocal recurrent selection. It is being explored in corn breeding and a little by poultry breeders and in swine. It seems unlikely to be profitable in such slowly breeding animals as cattle or sheep, but we can not be sure about that until we know more about how much heterosis can be gained by it. In this method, one selects wholly on the progeny test, but those progeny must be out of mates like those of the very line on which this line is being bred for future commercial use.

Even if reciprocal recurrent selection will not pay, making partially inbred lines for this purpose and then sorting out the ones among them which cross best might be profitable. At the moment, this seems even less likely to be successful than does reciprocal recurrent selection, but we can not be dogmatic about that until we know more surely the magnitude and causes of heterosis.

5. If the interactions between heredity and environment are important enough, breeding special breeds for special regions may be worth while. In sheep breeding, this has long been considered important, and much is written about some breeds being good for hill country, others better on arable land, others better in swampy regions, still others in semidesert regions, etc. We have not supposed that this is important in dairy cattle, although it has been considered almost axiomatic that the high-fat breeds are better suited to economic circumstances where the main product sold is butter, while the high-milk breeds are better suited where the main product sold is whole milk or cheese. In recent years, we have seen more suggestions that the breeds do not rank the same in all climates or under all systems of feeding. Should we concentrate more on breeding the animal to fit the local environment, so far as that can be done, and have it still fulfill its main purpose of producing fat and milk? Yet the overhead costs of producing a breed are moderately high. The numbers of animals needed must be large enough, and the need for special qualities in them acute enough, to more than meet these extra overhead costs.

Many of us used to say that the warm-blooded animals carry their own environment with them. Indeed, many and marvelous buffer mechanisms, in their blood or other physiology, do operate to hold their internal environment steady. But perhaps the limits on this physiological homeostasis are narrower than we generally think?

To achieve distinct success in breeding for local adaptability, introducing animals from regions with distinctly different climates or other conditions must cease almost completely.

Otherwise, the local race would never become very different genetically from the rest of its breed. The frequent backcrossing to the rest of the breed would continually jerk it back toward the average genetic composition of the breed and would tend to cancel all but the initial success in selecting for local adaptability. Our dairy breeds have never yet separated into such distinct local populations. Perhaps, after all, they have enough individual adaptability that we need not go far toward creating specialized local races? Perhaps . . . but many of us are not so sure as we were 20 yr. ago.

6. How to get more cows tested and to use the data while there is still time, will remain an important problem indefinitely in the future, just as it has always been. Central data processing, centralized testing, and other innovations in assembling and interpreting the data more quickly are certain to develop further. For genetic improvement, the important thing is to test the cows in those herds from which future bulls may come and to test the daughters of young bulls being proved. Testing cows in herds other than those may, of course, pay for economic reasons and for its usefulness in good farm management.

7. Sounder actuarial figures on such topics as expectation of life, death rates, percentages of barrenness, etc., would permit narrowing the rather wide confidence intervals which, at the moment, must surround such estimates as those of Robertson and Rendel. Knowing such norms more precisely would also permit keeping individual breeding plans more closely geared to the possible and to the probable. For instance, it is important to know whether, on the average, 100 cows of breeding age can have only 25 daughters per year grow up to freshen and enter the herd, or 30, or 35.

8. Making new breeds. The rules for probable success in making new breeds are becoming clearer, although much remains to be known. Economic success in that is more likely in regions or under circumstances where none of the existing breeds fit the requirements at all well; i.e., where the economic niche which needs filling is a large and nearly vacant one. The many new breeds of beef cattle along the Gulf Coast, in the last 30 yr., or the many new breeds of light horses, illustrate what might happen in dairy cattle but seems unlikely at present. The major rock on which foundered most of the earlier attempts to form new breeds seems to be that they had too narrow a genetic base and kept it narrow too long. But using at all times enough different sires, each fairly heavily, will require numbers large enough to put the enterprise beyond the reach of all but a few individual breeders. Cooperative effort might solve that and permit small operators to play a part, but such cooperation in the past has been wide enough and enduring enough to achieve its end only where the new breed filled well a big economic need (an important and nearly empty niche, as in making the American corn belt breeds of swine), so that the new mixture paid its way almost from the start.

9. Making new mutations. Experience gives little hope that the breeding value of cattle can be improved by forces such as X-rays or mutagenic chemicals. First of all, these things produce mutations only rarely. Second and more important, nearly all the mutations thus produced have been harmful. It is, I suppose, remotely possible that we may some day learn how to make certain kinds of mutations and not make others. If we ever learn how to make mutations to order, the proportion of useful ones among them no longer will be so vanishingly small. That, however, seems a remote prospect. Dairy cattle are such unsuitable material for studying mutagenic processes that persons interested in dairy cattle genetics will doubtless sit on the sidelines in this respect and watch the results with laboratory organisms until such time as those results suggest procedures which seem worth trying with dairy cattle.

## PROGENY TESTING METHODS IN EUROPE[1]

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The progress which can be made in the improvement of milk and butterfat yield in dairy cows depends to a large extent on the selection of males. Robertson and Rendel (12) estimated that in a closed population of 2,000 cows with the optimum structure, 43% of the total improvement arises from the breeding of young bulls from tested sires and 33% from selection of the same bulls on their dams' yield. The selection of dams of cows would contribute only 6% to the progress. Since about 60% of all heifer calves are needed for herd replacement, and many other things than the milk yield of the dam are decisive for whether a heifer calf is raised, the efficiency of female selection will always be low.

In Denmark, progeny testing of dairy bulls started in 1902, based on records from the cow

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testing associations, and since 1912 these tests have been carried out as dam-daughter comparisons. To avoid age correction of the milk records, the daughters' first records are compared with their dams' first records, the daughters' second record is compared to the second record of the dams, etc. The reports state the number of dam-daughter pairs, the average yield of the daughters (milk butterfat, and fat percentage), and the difference between the averages of the daughters and their dams. The errors involved in this type of progeny test arise mainly from (1) the environmental differences between herds, and (2) the changes in herd average yield from the time when the dam's record was made to the time of the daughter's record. When the bulls are used in artificial insemination, and their semen is spread at random on a large number of herds, the first type of errors will more or less cancel each other, but the second source may still be important.

In Sweden, an index was proposed by Hansson (2) for appraisal of the bulls' breeding value with regard to the fat percentage of the milk, based on the assumption that the daughters' butterfat percentage is intermediate to that of their dams and the sire's hereditary level. The same index was developed independently in the U.S.A. by Yapp (17). Since then, it has been used in several countries for milk and fat yield as well as for butterfat percentage, under the name of the Hansson-Yapp index or the equal parent index. It works fairly well for butterfat percentage, but it gives misleading results with regard to milk and butterfat yield when there have been pronounced environmental differences between dams and daughters.

Before the first world war a German extension specialist in cattle breeding, Peters (9), started to compare the daughters of a bull to their stable-mates. This idea was taken up in a modified form by von Patow (8), when he expressed the average daily yield of each cow during the calving interval in per cent of the contemporary herd average (Stalldurchschnitt). This idea of the contemporary herd average, or the average yield of the stable-mates, as a basis of comparison in the progeny test has been further elaborated in Great Britain, Sweden, and the U.S.A., to eliminate the errors due to environmental differences between herds and periods.

The rapid progress of artificial insemination in the northwest of Europe has greatly stimulated the interest in progeny testing. At the present time, about 95% of the dairy cows in Denmark are artificially inseminated, in Holland and Great Britain 60%, and in Sweden 40–45%. Progeny test for lethals or other undesirable recessive genes is not considered necessary in connection with artificial insemination, because each carrier will reveal himself anyhow at an early stage if the recessive gene has reached a frequency of a few per cent in the cow population for which he is used. The efforts are concentrated on progeny tests for the production characters of the animals.

The sources of differences between the averages of progeny groups may, for the purpose of discussion, be classified as follows:

I. Genetic differences:
   1. Between sires
   2. Between daughters of the same sire (sampling errors)
   3. Between dams of the daughters

II. Nongenetic differences:
   A. Randomly distributed (sampling errors): Environmental variations which are equally likely to affect any individual. Errors in measurement of the character.
   B. Causes of variation which are not randomly distributed but can be eliminated by corrections or standardization, e.g., age at calving, season of calving, and length of the milking interval.
   C. Environmental differences between progeny groups (feeding and management), mainly differences between herds and trend within herds.

The repeatability of the progeny test is a measure of its accuracy under certain conditions. If the variance between progeny groups has the following components: $G_s$ = genetic variance due to differences between sires, $C_s$ = environmental variance between progeny groups, and R = the variance due to randomly distributed causes (I: 2 and II: A), then the regression of future daughters on those tested, the repeatability of the progeny test, is

$$\frac{G_s}{G_s + C_s + R/_n} = \frac{n\,0.25\,h^2}{1+(n-1)\,(0.25h^2+c^2)}$$

where $n$ = the number of daughters, $h^2$ = the heritability of the trait, $c^2$ = the fraction of the variance which is due to nongenetic differences between the groups, and 0.25 = the coefficient of relationship between the paternal half sisters (random mating). It is assumed here that there is an environmental correlation ($c^2$) between the tested daughters of the same bull, but no such correlation between the tested daughters and future daughters, as would be true if each bull is tested in one herd and used later in any other herd of the breed. The repeatability of the progeny test increases with increasing number of daughters, but it decreases with rising value of $c^2$, as shown by the following figures; when $h^2 = 0.25$:

| | Number of daughters in progeny test | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 40 | 100 | 1,000 |
| $c^2 = 0$ | 0.25 | 0.40 | 0.57 | 0.73 | 0.96 | 0.99 |
| $c^2 = 0.15$ | 0.17 | 0.21 | 0.25 | 0.27 | 0.27 | 0.27 |
| $c^2 = 0.30$ | 0.13 | 0.15 | 0.17 | 0.17 | 0.17 | 0.17 |

For a reliable progeny test it is necessary to eliminate the environmental differences between progeny groups, i.e., the environmental correlation between half sibs. If the environmental correlation between half sibs ($c^2$) is large, the repeatability of the progeny test soon reaches a maximum value with increasing number of daughters. It is of no use to increase the number of progeny in the groups above 10 or 20, if they are not distributed on different environments, i.e., different herds. When the test is made in a single herd it applies only to this herd, or to other herds on the same environmental level.

It is shown in several investigations that the heritability of inter-herd variation in milk and butterfat yield is low, on an average 10–15%, Pirchner and Lush (10). Therefore, the herd differences can be disregarded and the progeny test based only on the deviations of the daughters' yield from stable-mates of the same age or from the contemporary herd average. For A.I. bulls that are tested on a large number of daughters, distributed at random on many herds, the simple daughter average can be used, but the deviation from contemporaries has the advantage that the variance within progeny groups (the error variance) is smaller. If the dams are selected, thus deviating from the herd average, a correction for their influence on the daughters' yield may be needed.

The purpose of the following review is to show the attempts which have been made in some of the European countries since the end of the war to solve the problems involved in the practical application of progeny testing to dairy bulls.

*The Danish progeny testing stations.* The station test of A.I. bulls was designed and started in 1945. In 1957–58 a total number of 102 bulls were tested at 33 different stations. Most of the testing stations are private farms where the owner has agreed to provide housing, feed, and care for the heifers on test, and in return he is getting the milk they produce. As a rule, two or more progeny groups are tested simultaneously at the same station. The Animal Research Institute at Copenhagen is supervising the work and appoints an assistant for each testing station. The assistant is in charge of all record-keeping throughout the testing period; half of his salary is paid by the state and half by the A.I. associations. As a rule, the owners of the heifers get a certain remuneration for placing the animals at the station for a year. In some cases, however, the A.I. associations buy the heifers, and sell them after the completion of the test. The rule for the station test may be summarized as follows:

1. A group of 18–20 daughters from each A.I. bull assigned for the test is brought to one of the testing stations about the first of September. At least 15 daughters must complete the test if the results are to be officially recognized. At delivery to the station the heifers should be pregnant and due to freshen between the first of October and the fifteenth of November, at an age of 27 to 33 mo. They are bred again at the first heat when 2 mo. have passed from calving.

2. The station test covers the first 10 mo. of the lactation period. There is a considerable variation, actually from 230 to 380 days, but the average for each group is almost without exception 304 days.

3. At the stations the animals are kept on a high level of feeding and management, and it is attempted to standardize this level for all stations as far as possible. Before calving the heifers are fed liberally, to equalize their condition at calving time, and after calving the ration is adjusted according to the milking ability of the individual cow. The cows are milked three times daily until the yield has decreased to a certain level, when the number of milkings is reduced to two.

4. During the stall feeding period the feed is weighed daily for each individual. The milk is weighed and the butterfat content determined on two consecutive days per week. The rate of milk flow during milking is determined on two days during the second to fifth months of lactation. The animals are weighed on arrival at the station, after calving, at the beginning of March, at the transfer to pasture, and at the end of the testing period. At the March weighing body measurements also are taken.

The main principles of the station test are (1) that the daughters of the bulls are chosen at random without considering the milk yield of their dams, (2) that they all freshen in the same season and approximately at the same age, and (3) that the records are made in a standardized environment. Comparisons between the progeny groups are based simply on their average records at the stations.

Analyses of the data from the Danish testing stations have shown that the attempts to eliminate various nongenetic differences between progeny groups have not been entirely successful. Johansson (4) found significant differences between the groups in regard to age at calving and length of the service period, and there were indications also of group differences in body development and condition of the heifers on arrival at the stations. However, the effect of these differences upon the milk yield is probably too slight to influence materially the testing results. More serious errors would arise from environmental differences between the groups during the period of test. That differences appear between stations and years is not surprising because variations in the quality of pasture and winter roughage can hardly be avoided, but it is suspected that environmental differences occur also between contemporary groups at the same station. One reason for the occurrence of such differences is, perhaps, that the heifers are placed in groups in the barn and not randomized.

Robertson and Mason (11) studied the repeatability of the station test in the field for 62 bulls

that had at least 80 tested daughters, divided on three different levels of herd average yield. The regression of the average milk yield of the field tested daughters on that of the station tested was for all herds 0.16 and for high herds 0.21 when calculated within regions. Hofmeyr (3) found that when the yield was calculated in the same way in the field as at the stations (250 days lactation), and on approximately the same number of daughters, and the differences between herds and years were eliminated, the regression of the field test on the station test was 0.23. When the field test comprised 34–67 daughters per bull and was based on the first yearly record, the regression on the station test was about 0.4. Thus, the repeatability of the station test in the field has been found to be rather low.

It would be expected that the nongenetic variation between individuals and progeny groups is lower at the stations than in the field, because the milk recording is more accurate at the stations, the effect of season of calving is practically eliminated, and each cow is fed individually in relation to its capacity of yield. It was surprising however, when Robertson and Mason (11) found that the variance between progeny groups was much higher at the stations than in the high yielding herds, whereas the variance within groups was lower. The difference in apparent heritability was, therefore, very large. Touchberry and Rottensten (15) estimated on the basis of the station data the apparent heritability of fat yield at 0.65 and from the field data at 0.20, after elimination of station, year, and herd differences. Hofmeyr found, however, that the correlation between the station tested daughters and their field tested dams was higher than that between the field tested half sisters (of the station heifers) and their field tested dams, 0.178 versus 0.134, which would seem to indicate that the station test of the individual cows is more accurate than the field test.

Robertson and Mason reached the conclusion that the same accuracy with regard to milk yield can be obtained by the field test on 30 daughters as by the station test on 18 daughters. The station test has, however, the advantage that it easily and for a low additional cost can be extended to other traits than milk and butterfat yield, e.g., the rate of milk flow during milking, the feed consumption of the animals, body size and conformation, the intensity and duration of heat, etc. The randomization of environment can be improved, even if it is impossible to make it perfect. The main disadvantage of the station test is the high cost involved.

At the completion of the progeny test at the Danish stations in 1957–58, 59% of the bulls were below 6 yr. of age, 32% were 6–7 yr., and 9% were 7–8 yr. old. Under A.I. conditions it should be possible to complete a field test on 50 or more daughters when the bulls are about 5½ yr. old.

The amount of culling which takes place among the station tested bulls in Denmark immediately after the completion of the test is slight, but the superior bulls are used to a much greater extent than the average or poorer bulls.

*Sweden.* Since 1953 the progeny testing of dairy bulls is centralized to the Wiad Research Station (Institute of Animal Breeding) and financed by the state. A Committee for Progeny Testing (NAT), with representatives for the breeders' and the A.I. associations, is responsible for the work. Records of the individual cows are delivered monthly by the cow testers to the progeny testing center, and the average yield of each herd is calculated and reported with 4-mo. interval. In each county there is a cow testing supervisor who is responsible for prompt delivery of the data.

The first progeny test of a bull is made as soon as he has at least ten daughters with record for the first lactation yield (305 days). When heifers which have completed only 46–304 days of lactation are culled from the herds, their records are used after conversion to 305-day yield. This is done to avoid selection within the progeny groups. The frequency of culling among the first calvers before they have completed their first 305-day lactation varies between the breeds; for Swedish Red and White Cattle 10–12% and for the Swedish Friesian 15–20%.

The average first lactation yield of milk and butterfat is calculated for all the daughters of the bull and corrected to yield at a standard age of 28 mo. at first calving. The milk yield is also converted to a standard fat content of 4%. No dam-daughter comparison is made, but the daughters' average yield of fat-corrected milk is compared to the corresponding yield of daughters of other bulls in herds on the same production level as that where members of the particular progeny group were raised and produced. The comparative yield of heifers in herds on the same production level is calculated from the regression of first calvers on their contemporary herd average. For the two most important dairy breeds the figures used for these computations are as follows:

| | Regression of first lactation yield on herd av. | Herd av. for first lactation | Herds' average for cows of all ages |
|---|---|---|---|
| | | *(kg. milk)* | |
| Swedish Red and White Cattle | 0.651 | 3,468 | 4,121 |
| Swedish Friesian | 0.620 | 3,670 | 4,587 |

For a Swedish Red and White herd, with an average yield of 5,005 kg. 4% milk, the corresponding average for the first lactation yield is 3,468 + 0.651 (5,005-4,121) = 4,054 kg. milk. The average yield of the bull's daughters is expressed in per cent of the comparative yield. If the daughter's average is 4,400 kg. milk, the

relative yield of the daughter group is 108.5. The difference between this relative yield and 100 is adjusted for the number of daughters on which the test is made. The correction factor for progeny groups of ten daughters is the same for bulls in A.I. and natural service, 0.5, but whereas the factor rises curvilinearly to 1.00 for 50 (or more) A.I. daughters distributed on many herds, it rises only to 0.7 for bulls in natural service, and there is no further rise for increasing number of daughters. For 20 A.I. daughters, e.g., the correction factor is 0.77 and for the same number in natural service 0.62. Applied to the previously stated relative figure 108.5, the result for 20 A.I. daughters will be $0.77\ (108.5 - 100) + 100 = 106.5$. This is the relative value for milk yield of the progeny group.

The reports of the progeny test contain the following information: the number of daughters included in the test with 305 and with 46–304 day yield, the average calving age of the daughters, their average yield of milk with 4% fat, corrected to 28 mo. calving age, the corresponding average for daughters of other bulls in herds on the same production level, the relative milk yield of the progeny group, and the average fat percentage of the daughters' milk and the corresponding average for their herds. From these data, the breeders have to draw their own conclusions.

The progeny test is repeated when the bull has twice as many daughters with production records as those used in the first (or previous) test. Special progeny tests of bulls, taking also the yield of the dams into consideration, are made upon the breeders' request and at their own expense. The regular tests are free of charge.

The main principles of this progeny test are: (1) The test is based on the first lactation yield of all tested daughters of the bull, (2) the individual records are not age-corrected, but the group average is corrected to a standard age at first calving of 28 mo., (3) the relative milk yield of the progeny group is expressed by a percentage figure, where the yield of first calvers in herds on the same level of production is the basis of comparison, and (4) the relative yield of progeny groups of less than 50 heifers is regressed towards the mean (100), depending on the number of heifers in the group. The relative yield of daughters of bulls in natural service is always regressed towards the mean, even when the group contains 50 or more heifers.

The Swedish progeny testing scheme is very similar to the one used in New Zealand. The main differences are that in New Zealand all records are corrected to mature yield at 4–9 yr. of age, and that no relative yield is calculated but rather the difference between the average of the progeny and that of daughters of other bulls in herds on the same production level.

The average age of a random sample of 120 A.I. bulls tested in Sweden during 1958 was 6⅓ yr. at the completion of the test. The corresponding age for 150 bulls in natural service was 7½ yr.

Two bull testing stations were organized in Sweden some years ago, one for Swedish Friesian bulls and one for Red and White bulls. Only the last-mentioned station is still in operation.

*Norway.* In 1955, a new type of field progeny test was started, called the Hedmark system after the county where the test was first practiced. An area with dense cow population and a high percentage of tested cows is chosen as test area for the bulls at an A.I. center. In Hedmark county, 63% of the cows are production tested. Within this area practically all the cows are inseminated during a limited season (December-January) by semen from the young bulls assigned for the progeny testing. To make an effective selection possible, about five times as many young bulls are used for these inseminations as the number of bulls needed after completion of the progeny test. Each bull is used for so many cows that 75–100 daughters can be expected to be raised and complete their first lactation. The daughters stay on the farm where they are born, and their milk yield and butterfat percentage is tested according to the regular scheme once every third week; in addition, also, the protein content of the milk is determined. The milking characters of the cows (rate of milk flow and milking time) are measured by a special assistant travelling from one farm to another. To reduce the errors in measurement of the milking time and the rate of milk flow, the milking machines on each farm are adjusted to operate at the same pulsation rate and the same vacuum level. The special assistant is also scoring the cows for conformation, particularly with regard to udder and teats, and he takes a series of body measurements. He is supposed to visit each farm two or three times during the first half of the lactation period of the daughters. The milk yield of the cows has been recorded for 200 and 300 days of lactation, but it is proposed to record it also for the first 100 days to make an early preliminary test possible, Skjervold and Sandvik (14).

As soon as the necessary data are available, the following averages are calculated for the progeny groups: the milk yield (actual and corrected to 4% fat), the fat and protein content of the milk, the milking rate and milking time (corrected for yield), and the average scores for udder and body conformation. The contemporary average yield of the daughters' herds is also calculated, but since the number of daughters always is large, and the daughters are distributed at random on at least some 40–50 herds, the herd average is not used for the calculation of any bull index.

The following advantages of the Norwegian progeny testing scheme for A.I. bulls may be pointed out: (1) All the daughters of the bulls,

tested in a certain area, freshen at approximately 2 yr. of age (extreme variants are excluded) and always in the same season. The daughters are spread at random on so many farms that the effects of herd differences in feeding and management are eliminated. Therefore, no corrections for age, season of calving, or herd environment are needed. (2) There are no possibilities of selection of the daughters on their own yield and little possibility of selection on the dams' yield. (3) Even with the supplementary tests of the daughters, made by special assistants, which can be extended also to other characters, e.g., reproductive efficiency, the cost per progeny tested bull of this field test is only about 20% of that at the Danish testing stations.

*Finland.* The Department of Animal Breeding at the Agricultural Research Center, Dickursby, is in charge of the progeny testing, which is carried out when a bull in natural service has at least 10, and an A.I. bull at least 20, daughters with yearly milk records. No records of cows above 6 yr. of age are used. The average milk yield of the daughters is expressed in per cent of the contemporary average of the herd (or herds), and for comparison also the average yield of all progeny groups of the same age is calculated in per cent of their contemporary herd average. The difference between these two percentages is an expression for the breeding value of the bull in comparison to the breed average. The average fat percentage of the daughters' milk and the corresponding herd averages for milk and fat percentage are also calculated and stated in the reports, Varo (16).

*Great Britain.* The Milk Marketing Board is in charge of the progeny testing of A.I. bulls in England and Wales. The test, originally devised by Dr. Alan Robertson, is based on the first lactation yield (305 days) of the daughters of the bull and the corresponding yield of their contemporary stable-mates, Mason (6). In each herd where the bull has daughters with records that can be compared to those of contemporaries, the average yields of the daughters ($\overline{Y}$) and the contemporaries ($\overline{A}_Y$) are calculated, and the difference between the two averages is weighted for the number of heifers in the groups. The weighting factor ($w$) is the harmonic mean $\frac{n_1 n_2}{n_1 + n_2}$ of the number of daughters ($n_1$) and the number of contemporaries ($n_2$) in the herd. All these weighted differences for all the herds where comparisons are made are added, and the sum is divided by the sum of the weighting factors: $\frac{\Sigma(\overline{Y}-\overline{A}_Y)w}{\Sigma w}$, in the following abbreviated to $D_w$. When a bull is progeny tested, the value of $\Sigma w$ should be at least 20 which, on an average, corresponds to about 30 tested daughters. The weighted difference between the bull's daughters and their contemporaries is adjusted for the number of daughters by multiplication with the factor $b = \frac{0.25h^2\Sigma w}{1+(\Sigma w-1)0.25h^2}$ (the regression of future daughters on those tested). When $h^2 = 0.25$ the value of $b = \frac{\Sigma w}{\Sigma w + 15}$. The estimate of the breeding value of the bull is $2b\ Dw + \overline{A}$ in relation to all first calvers ($\overline{A}$) in the herds where the test is made. For a comparison with the population as a whole, 0.2 ($\overline{A} - \overline{P}$) is added ($\overline{P}$ = the breed average for first calvers), assuming that the heritability of the interherd variation is 0.2. The relative breeding value (R.B.V.) of the bull within his breed is expressed as

$$\text{R.B.V.} = \frac{[2b\ D_w + 0.2\ (\overline{A} - \overline{P}) + \overline{P}]\ 100}{\overline{P}}$$

The addition of 0.2 ($\overline{A} - \overline{P}$) is probably unnecessary under A.I. conditions where the genetic differences between the herds tend to be small. When the test is made on at least 30 daughters, the error introduced by disregarding the dams is probably also rather small.

Robertson *et al.* (13) point out that the main advantages with this method of progeny testing are (1) that age corrections are avoided, since the daughters of the bull and their stablemates, on an average, start their first lactation at the same age, and (2) that each daughter is compared to contemporaries in the same herd, i.e., on the same level of feeding and management, which would seem to be the most rational method of comparison. As a disadvantage may be mentioned that the method does not make use of all the available information about contemporaries in the herds, since only contemporary first calvers are considered. For big herds this is of no importance, but for herds with less than 20–30 cows the situation is different; in many small herds there may not be any contemporary daughters of other bulls to compare with.

Dr. Edwards, Milk Marketing Board, has supplied the following information on the organization of the progeny test of A.I. bulls. Semen is collected from the young bulls when they have reached 15 mo. of age, on an average, for insemination of about 500 cows in production tested herds within a limited period of time, which may vary from a few weeks to 6 or 8 mo., depending on breed and area. The regular progeny test can then be made when the bulls are 6 to 6½ yr. old. To make earlier culling possible, a preliminary test is made on the milk yield during the first 180 days after calving. On an average, about eight out of ten bulls entering the test are still active when the test is complete and it is attempted to cull four out of five tested bulls. The average useful life of the selected tested bulls is estimated to 4 to 5 yr. So far, the use of the young bulls between the insemination of 500 cows and com-

pletion of the test has not been limited, but it is planned to lay off completely after about 1,000 cows.

*Holland.* All bulls are progeny tested when at least 15 daughters have a complete lactation record. When records are available for 15 dam-daughter pairs of the same age, a dam-daughter comparison is made. No indices are calculated. The average yield of milk and butterfat, and the butterfat percentage for the dam and daughter groups, are published in the herd books, together with the number of days in milk. In some parts of Holland the protein content of the milk is also determined for each cow on each testing day. Where this is the case, the daughters' average protein content of the milk is calculated.

In addition, a random sample of daughters of all A.I. bulls, as well as daughters of the best bulls used in natural service, are inspected by a special committee and judged for conformation. This inspection is made twice, the first time on the pregnant heifers and the second time when they are in milk. At the second inspection the judging of the udder is an important item. At these occasions the daughters are also compared to their dams.

No special tests are made of the bulls for lethals or other undesirable recessive genes, but segregation of defects is recorded and the information on such cases is made available to the breeders. All members of the Friesian herd book are obliged to report to the herd book office on all calves born by registered cows. Detailed records on the fate of all calves born are kept also in connection with the A.I. work, which now comprises 60% of the total cow population in Holland.

In 1952, some A.I. associations started an intensified scheme of progeny inspection comprising 100 randomly chosen daughters yearly of each bull. Here attention is paid also to the milking characteristics, fertility, etc. of the cows.

*West-Germany.* The scheme for progeny testing varies somewhat from one province to another. The most common method is, however, the dam-daughter comparison (TMV) based on the relative yield compared to the herd average. The yield of each cow per record year (12 mo.) is expressed in per cent of the contemporary average of all the cows in the herd. When the herds are very small, the average yield for a group of herds may be used for comparison. Each daughter and her dam enter this comparison with the same number of records started at approximately the same age. For first calvers the yield during the first 305 days may be used instead of the 12 mo. record. The average fat percentage of the daughter group is compared directly with that of their dams without reference to the herd average, Langlet (5).

Reports on the results of the progeny test contain information on the number of daughters and records on which the test is made, the average milk and butterfat yield of the daughters, and the contemporary average of their herds, the relative yield of the daughters (in per cent of the herd average), and their average fat percentage. The same figures are presented also for the dams. In addition, the production average for all the daughters of the bull is stated, including those where no dam-daughter comparison could be made. In some cases also an Erbgitter (hereditary grid) may be presented, showing how the dam-daughter pairs arrange themselves in a correlation table.

A preliminary progeny test may be made on the yield during the first 200 (or in some cases 100) days of lactation of the daughters, to obtain information on the breeding value of the bulls as early as possible.

*Switzerland.* The bulls are tested on the average yield of all their daughters as well as on a daughter-dam comparison to the extent this comparison can be made. The actual yield is evaluated in relation to the average yield of the herds in the breeding asociation. The male and female progeny are also judged for conformation by special experts. For this purpose the progenies are gathered at a central place where they are arranged according to age and sex.

## SUMMARY

A common feature of the European methods for progeny testing of bulls is the attempts made to eliminate the effect of environmental differences between progeny groups. In Denmark, special testing stations were inaugurated for this purpose. The station method is, however, rather expensive. The cost per tested bull has been about $1,500 in recent years; at one Swedish station the average cost was $2,300. The high cost is probably the main reason why the station test has gained little ground outside of Denmark. At the present time two stations are in operation in Great Britain, one in Germany, and one in Sweden.

When the progeny test is based on field records, the average yield of the herd, or that of the stable-mates, is used as a basis of comparison. In Germany, the yield of each individual cow is expressed in per cent of the contemporary herd average, and the relative yield of the daughters is compared to that of their dams. In Sweden, the average first lactation yield of the daughters is expressed in per cent of the corresponding average for all first calvers in herds on the same production level as the daughters' herds. After regression towards the mean (100%), in cases of small samples of daughters, this relative figure is used for comparison between progeny groups. In Great Britain, the first lactation records of the daughters of the bull are compared to those of their stable-mates of the same age, the difference within each herd is weighted according to the numbers of daughters and stable-mates, the total difference is regressed for small numbers of comparisons, and the bull's relative breeding

value is expressed in per cent of the breed average. Correction factors for age are avoided by comparing only first lactation records. The use of the first lactation only has the advantage that the progeny test of the bulls can be completed early.

The British (Milk Marketing Board) method has the disadvantage that in small herds there may not be any contemporary daughters of other bulls to compare with. When the herds are so large that sufficient number of contemporaries, calving at approximately the same age and in the same season, can be found, it seems likely, however, that this method for field progeny test will give more accurate results than any other method which has been used so far.

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# OUR INDUSTRY TODAY

## ITALIAN SOFT CHEESE ON THE NEW YORK MARKET[1]

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Italian soft cheese has gained more popularity among consumers in the past 10 yr. and has created more controversy among manufacturers than any other cheese type on the American scene.

New York and Wisconsin have shared in the surprising growth of these cheeses and it would serve their interests to continue this sharing. A step in the right direction in holding such an advantage is that of learning more about the various Italian soft cheeses and of understanding the problems of the manufacturer not only in Wisconsin but in other regions.

*Types of cheese in the Northeast.* Three important Italian soft cheeses in the Northeast are Mozzarella, Ricotta, and Impastata. Years ago the New York markets catered to Italian soft cheese made from whey and skimmilk. Now most of this cheese is made from 2% fat milk or from whole milk. For example, Ricotta cheese in the New York market practically always is produced from whole milk. In the Midwest, however, Ricotta cheese is that made from whey and skimmilk. The trend toward making Italian soft cheese from higher-fat milk is diametrically opposite to that of fluid milk practices, and it is indeed welcomed in areas of surplus whole milk.

*Principles and descriptions.* Mozzarella cheese manufactured in the New York area usually is formed into large curd blocks at upstate factories without any cooking of the curds and without much acid development. Acid develops in a day or two when the blocks are brought out from the cold room to warmer temperatures. At optimum acid development, the curd is stretched in hot water. The finished cheese, lightly salted, is white, soft, with a very lively surface sheen. This cheese by Midwestern standards has a high moisture content.

Mozzarella cheese is used for direct eating, for varied Italian dishes including lasagna, and for the layering of pizza pies. To the supermarket consumer Mozzarella appears as a small round parcel, perhaps not more than 0.5 lb. in weight. Not much difference exists between Mozzarella and Scarmosa, except that the latter has a small head around which a string can be tied. In establishments catering to pizza pie clients, rectangular blocks of Mozzarella cheese called cukes are often used.

In New York, common practice has been to turn out the rough curd blocks at the upstate factory; to ship these curd blocks iced to Lattichini, or milk stores, in New York City. In these stores the curd is acid-ripened, then heat processed to the order of the customer. The advent of chain store supermarkets is forcing the Lattichini out and along with it this individualistic method of finishing the Mozzarella cheese. More and more the cheese is produced in its final form in the upstate New York factories and sold in chain stores.

Ricotta cheese, or recooked cheese, when made from whole milk and consumed fresh with its delicate texture and succulent nutty flavor, has more admirable qualities than creamed cottage cheese, if the two can be compared. One other admirable quality is its yield of 23%, the highest of any cheese.

In Eastern Ricotta cheese making, whole milk is acidified to about 0.3% lactic acid with enough starter and heated in large kettles to 176° F., although this temperature varies with the season. At this high temperature the proteins separate as a curd, entrap the fat, and float to the surface, where the mass is scooped off as fresh Ricotta cheese. Because all of the proteins of the milk are precipitated by this procedure, the very high yield is achieved.

Ricotta cheese is eaten directly or utilized in the preparation of certain dishes, including ravioli, and as creamed-whipped fillings for dessert. Backed by high quality, packaged in the right container, and sold with vigorous advertising, whole milk Ricotta should have a happy future in all areas of the United States.

Impastata cheese, or whipped pasta, has found an important niche among bakers for pastry fillings, and among cooks for the preparation of such dishes as ravioli. It is a cheese made from whole milk or 2% fat milk, embodying many of the principles of Ricotta cheese. The cheese is drier than Ricotta and for certain uses this is preferable. The preparation of Impastata is carried out in ordinary cheese vats, instead of in large kettles, and instead of the curd floating to the surface as in Ricotta, it is deliberately driven to the bottom of the vat. This is accomplished by controlled agitation of the curd and by cooking milk acidified more highly than that for Ricotta. The yield of this cheese is about 20%.

*Some technical problems.* Technical problems of various types remain to be solved in the Italian soft cheese industry. For example, Mozzarella cheese, at least for Eastern markets, is sold as a white product. But to pre-

[1] Address delivered at Wisconsin Dairy Manufacturers' Conference, University of Wisconsin, Madison, January 28, 1960.

pare a white Mozzarella cheese is often difficult throughout the year because milk varies in color. Homogenization of the milk for Mozzarella cheese, or the use of a bleaching compound, benzyl peroxide, is advocated. In either case, the stretching quality of the cheese is impaired with such treatments. A practical solution for producing a white product throughout the course of the year is not too clear, but there is continuing interest in the problem.

Another technical problem deals with mechanizing Ricotta cheese making. To a very large degree this cheese requires many manual movements, especially in the removal of the curd from the surface of the kettles. Perhaps five or six men are engaged for every three kettles of cheese. A desludging separator used in the preparation of Neufchatel and Baker's cheese was applied to the removal of Ricotta curd from the kettles. Though removal physically was possible, the resulting cheese, according to the manufacturer at whose plant it was applied, was too wet and without the proper body or texture.

*New York state standards for Italian cheese.* New York has standards of identity for Mozzarella and Ricotta cheese. None exists for Impastata. At the present time, no federal standards for any of these three cheeses have been promulgated, and it is around this subject that the vortex of controversy swirls and has been swirling for the past 3 yr.

Mozzarella and Ricotta cheese sold in New York State, including New York City, must be manufactured in a manner specifically outlined by the State. It is somewhat of a mystery how any one can supervise the manufacture of a certain cheese to conform to a legal standard, but New York manufacturers are particularly sensitive on this point. Either Mozzarella or Ricotta cheese can be made from 2% fat milk or whole milk according to New York State law. If manufactured from whole milk, Mozzarella must have not less than 52 and not more than 58% moisture, and the fat content must be not less than 18%. Ricotta cheese made from whole milk must have at least 11% fat and not more than 80% moisture.

Conversations with Wisconsin people indicate that it is the high moisture content, among other things, which makes cheese manufacturers in the Midwest look with dismay at the New York State standards. With so much water required in a cheese, is it possible to transport a high-moisture Wisconsin Mozzarella cheese over 1,000 miles to New York City and still maintain good keeping quality? It is possible. Keeping quality of cheese is not wholly a matter of the moisture content. It is concerned, too, with the number and types of microorganisms in the cheese.

*Keeping quality of Italian soft cheese.* In the past 4 yr., Italian soft cheese samples from all areas of the country have been examined for spoilage organisms at our laboratory. Generally speaking, although there are exceptions, microbial counts of Italian soft cheese were high and the keeping quality was low. The high counts are attracting more than casual attention from law enforcement bodies. Soon, perhaps, numerical standards for certain types of microorganisms in soft unripened cheese may be established, both by government and state bodies. In an indirect way, establishment of such standards may be of great help to the industry because of consumer packaging. Such packaging of Italian soft cheese demands low counts of certain microorganisms; otherwise, many of these packages may be blown up and losses to manufacturers can be high.

Most spoilage in Italian soft cheese is due to yeast and mold. Coliform bacteria, however, are responsible in some of the cheeses. These microorganisms appear in the cheese as a post-contamination, and have no business being there. For example, a commercial Mozzarella cheese picked up at random some time ago contained 50,000 yeasts and molds per gram, whereas freshly packaged commercial Ricotta cheese sampled directly at a factory had a coliform count of 700, and a yeast and mold count of 5,000 per gram. It is interesting to note that this cheese was heated in the kettle to almost 180° F. for 30 min.

Any cheese to be shipped long distances must have a low number of spoilage organisms. To show that such shipment is possible, last summer in Italy a cheese factory daily transported Mozzarella cheese with a high moisture content from its Milan area factory all the way to Sicily in good condition, during the month of August. The keeping quality even of a high-moisture cheese can be maintained with strict standards of sanitation.

*Classification of Italian soft cheese.* The Italian soft cheese industry is waiting for the decision of our government as to standards of identity for Mozzarella cheese. Is it to be a Mozzarella cheese or is it to be a Pizza cheese? Later, a similar situation perhaps will arise with reference to Ricotta or Ricotone. In the New York area Ricotone cheese is that same type called Ricotta in the Midwest. Official hearings for the standard of identity for Mozzarella cheese are over, and anything said here will not influence the forthcoming decision. Still, this decision may be delayed for a long while. Perhaps enough time exists even now for the industry to settle the issues by themselves. While the cheese industry remains split on this issue, the American consumer goes along very poorly informed on the merits of various types of Italian soft cheese. For example, what is a true Mozzarella cheese? If we use the argument that Mozzarella should be that made in Italy many hundreds of years ago, then buffalo would be ranging through Wisconsin and New York, because for many years, in Italy, Mozzarella was made from buffalo milk.

Even today a certain proportion of the cheese in Italy is from buffalo milk. Perhaps a better criterion to use is to identify American Mozzarella with the Mozzarella cheese of modern Italy. But this cheese appears as different things to various travellers from the States.

In August, 1959, the writer traveled to Italy and spent some time with the research people at the National Cheese Experiment Station in Lodi, visited a number of Italian factories making Mozzarella, and finally visited various stores.

The Italian cheese made in modern Italy is mostly from pasteurized cows' milk, acidified with *S. lactis* starters generally. It is soft, white, and often packaged in Cryovac. According to Professor Carbone, Director of Research at Lodi, modern Mozzarella cheese has a moisture content of about 55%. There may be other types of Mozzarella cheese, but these were not evident. It was stated that a slightly drier cheese was sometimes sold to restaurants for pizza pie, but was not called Mozzarella cheese. It is interesting to state that Mr. A. Locatelli of the Locatelli Cheese Company is making in his new Rome plant this same Mozzarella cheese from Enterococci starter of the DK type.

After considering all aspects of the question, what is wrong with having two distinctive types of cheese in the Mozzarella line? Why not have a Mozzarella cheese and a Pizza cheese? Just as the car industry has found it profitable to make many types of cars within their own lines, it would seem to me that the Italian soft cheese industry could benefit similarly and make available to the consumer a group of very distinct cheeses within a classification. Surely, total sales of Italian soft cheese will increase if the consumer is given an opportunity for a wider choice.

If the entire Italian soft cheese industry wishes to prosper, it must form in the consumer's mind a picture of distinctive qualities in Mozzarella and Pizza cheese and later Ricotta and Ricotone cheese. This will never be accomplished by prolonged and bitter controversies, but only by a better understanding of all problems.

## THE DAIRY INDUSTRY IN THE U.S.S.R.[1]

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Dairy industry in the U.S.S.R. is forging ahead. Progress is being made on all fronts. There has been a great increase in the number of dairy cattle in Russia during recent years, and the productivity per cow is increasing rapidly. True, from detailed studies, we are not at all sure how accurate their figures are; but, based on recent reports, real progress is being made in the production, processing, and distribution of milk and other dairy products.

At the same time, it is well to remember that, based on the best available comparative report of work required in the U.S.S.R. and in the U.S.A., the average Russian has to work about 4½ times as long to buy a quart of milk as does an American. He must work 33 min. to buy a quart of milk as compared with 7 min. in the U.S.A. If the Russian wants to buy a pound of butter, he must work for 3 hr. and 20 min., as compared with about 20 min. in the U.S.A. A pound of beef requires 1 hr. and 31 min. of worktime as compared with 22 min. in the U.S.A. (18).

During August and September of 1958 it was the privilege of the authors to spend 30 days traveling and studying agriculture in the Soviet Union. They were part of a delegation of six that had gone to the U.S.S.R. as part of the exchanges in cultural, technical, and educational fields which had been functioning during 1958 and 1959.

While in Russia, the authors had an opportunity to study the over-all pattern of the dairy industry. They talked with dairy scientists, visited state and collective farms, especially dairy farms, and saw the distribution of milk through local markets and stores. A special visit was made to one of the five major milk combines, located in Moscow. At a number of research institutes, progress in dairy science was observed.

[1] The authors were members of a six-man exchange delegation to the Soviet Union in 1958.

DAIRY FARMING

While traveling through the U.S.S.R., several dairies were visited in the Ukraine and in the Newlands of Siberia, where milk production is encouraged greatly.

Dr. Lazar Volin of the USDA, a member of the Farm Economics Exchange Delegation in 1959 has reported that in May, 1957, Khrushchev had initiated a new agricultural drive in the U.S.S.R. in which he announced the intention of the Soviets to surpass the United States in per capita production of milk and meat. As far as milk is concerned, Khrushchev believed they could accomplish this goal in 1958. Therefore, it was no particular surprise that official reports were released in Russia in January, 1959, to this effect.

On January 16, 1959, the New York Times reported that the Soviets had announced that their 1958 production exceeded that of the United States (2). However, their per capita production lags behind the United States because of their greater population—approximately 210 million, compared with our 178 million.

Figures 1, 2, and 3 show comparative figures

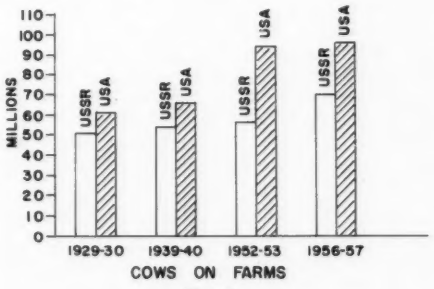


FIG. 1.

as they have been made available through various sources (11), (13), (19) as to productivity of the dairy industry in the U.S.S.R. and in the U.S.A. When it comes to the individual production of cows or dairies, it is quite a different story. In 1958, U.S. dairy cows produced an annual average of 6,330 lb. of milk per cow. For the same year, Russia's own figures indicate that they averaged about 4,000 lb. of milk per cow on collective farms. They claimed that their production per cow on privately owned farms was only 3,625 lb. per cow (13).

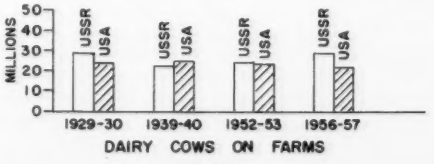


FIG. 2.

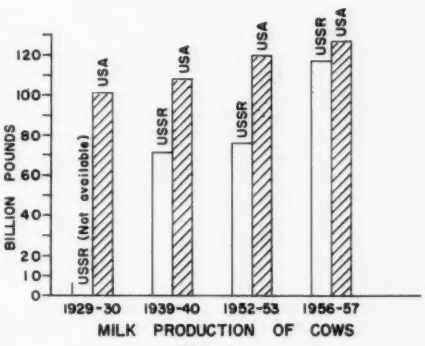


FIG. 3.

This is misleading because there are probably no large privately owned dairies. The privately owned cows are held as individual animals by a family, principally for family needs. As of October 1, 1956, of 30.9 million cows in the Soviet Union, 37.1% were on collective farms, 6.4% on state farms, and 56.5% were privately owned, principally by collective farm workers (16). There is a reason for this, which will be elaborated on later.

Khrushchev stated in December (11) that the production of one centner (220.5 lb.) of milk required 9.9 man-hours on Soviet state farms and 14.7 on collective farms. This compared with 4.7 man-hours on U.S. farms.

*State organization.* As background, one needs to realize that the Soviets are still making a hard drive to organize state farms in preference to collective farms, and also to get people to give up their private cows for inclusion in the larger, more efficient dairy herds. As one inducement to get individual owners to give up their cows, collective farms sell milk to the workers at a much lower price than the state pays the farms for their milk. This is meant to convince the individual owner that he would contribute more in the way of productivity of the large herd, rather than with his privately owned cow.

The government discourages raising animals on the family plots because it is an inefficient use of labor. The family plot is about 1 acre or less in size. There are about 5,800 state farms and 78,000 collective farms in the Soviet Union. About 50% of the farms are electrified. Collective farms vary from 2,000 to 40,000 acres, with the average being about 15,000 acres (with 4,200 acres in cropland) and occupy 70% of the land.

*Barn construction and facilities.* The dairy barn is typically a one-story structure with feed and alleyways of concrete or packed earth, with wood or steel stanchions, and a wood platform for the cows. Feed carts and water cups were used in some areas. Feed and straw are stored on the ground level, often

outside the main barn. Ensilage is usually placed in a horizontal underground silo built of brick, concrete, stone, or soil, although there appears to be a trend to use above-ground horizontal silos. These silos are about 5 m. in diameter and 5 m. deep, or 5 m. deep and 5 m. wide, with the length variable. Temporary milking barns are used for the cows in the summer. They are principally of pole-type construction. Electricity is usually furnished to these units by stationary diesel-electric generators. It is claimed there are 1,000 six-cow milking parlors. One outdoor milking parlor was seen.

Beet tops and other crop by-products are used for silage. Corn with the ears removed is used for fodder and silage. Special harvesting equipment is available for removing ears and chopping stalks in one operation. In some areas, green forage was chopped and fed at the barn. Green forage is supplied early in the spring in the form of winter rye, silage corn, and sweet lupine, and supplemented with hay and straw.

*Breeds of cattle and management.* There is one fundamental reason why it would be virtually impossible for the Soviet Union in the very near future to equal the production per cow of that in the United States. Most of the cows used for dairy purposes in the U.S.S.R. are dual-purpose animals. They are beefy and apparently have been selected for meat and milk. Some animals are multi-purpose—meat, milk, and beast of burden. There are many different breeds of cows. Animals are imported and crossed with native cattle. The identification of a new breed is based on the area of breeding. The Simenthal cow is the most popular and resembles the Guernsey in color. Other popular breeds are the Ukrainian Red Steppe, the Shorthorn, the Kholmozorsk, and the Holstein-Friesian.

Characteristics of four dominant breeds of cattle are as follows: (8) (9)

| Breed | Weight | Milk | Average butterfat |
|---|---|---|---|
| | *(lb.)* | *(lb. per year)* | *(%)* |
| Simenthal | 1,450 to 1,650 | 11,000 to 12,750 | 3.8 to 4.1 |
| Red Steppe | 1,100 to 1,200 | 8,800 to 11,000 | 3.6 to 4.1 |
| Black Spotted (black and white) | 1,200 to 1,450 | 10,000 to 12,000 | 3.5 to 3.8 |
| Lebedinsky | 1,300 to 1,500 | 9,500 to 11,500 | 3.8 to 4.1 |

Other breeds are known as Besterzhev, Altan, Kostroma, Polish Red Cattle, Ukraine Red Cattle, Kurgan, Tagil, Red Gorbatov, and Brown Latvian.

Apparently, most cows are milked three times per day (at 5 A.M., 12 NOON, and 8 P.M.). Milkmaids work four days and then have one day off. The pay for the milkmaids at the Telman Collective was about 1,000 rubles[2] per month plus some milk. The milkmaids are responsible for feeding, caring, and milking the cows. The milking machines were of the former DeLaval type, with the inflation ring in the teat cup. Aluminum milk pails predominated.

Artificial insemination is used rather widely. In fact, it is reputed to be used with 75% of all dairy cattle. Free artificial insemination is provided by the state to all collective and state farms and to the privately owned cattle. It is widely used by veterinarians not only with cattle but with sheep, horses, and to some extent swine. It is obligatory in herds where trichinosis and brucellosis exist.

### GENERAL IMPRESSIONS OF AGRICULTURE

The U.S.S.R. comprises a land of enormous physical and human resources. It occupies almost 1/6 of the total land in the world—about five billion acres. One-third of Russian land is forested, and makes up about 18% of the world's forest land. Less than 1/10 of its land is cropped, and much of its land is either swampy or lacks adequate moisture.

The majority of the cropland lies farther north than the United States. Today, the Soviets are farming a few million acres less than a half-billion acres of cropland to feed their 210 million people. This compares with approximately 360 million acres to care for 178 million people in the U.S.A.

Russia has ample farm land, and huge acreages have been brought under cultivation during the last 5 yr. However, the growing season is short. Women do a great deal of the farm work, and almost all of the land is in large collective and state farms, with small plots of an acre or so being allocated to families to be used as kitchen plots behind their dwellings.

*Problems.* Traditionally, Russia has had difficulty in adequately feeding its people. This is the basic incentive in attempts to develop large collective and state farms. The idea was to greatly increase mechanization and efficiency of food production. Although great progress has been made in food production in the Soviet, it is significant that there is believed to be no more livestock in the U.S.S.R. today than there was prior to the Revolution in 1917, and about half of the livestock is in the hands of individuals rather than being owned by the large farms.

[2] 1 ruble = 100 kopeks = 20 cents (official exchange) = 10 cents (tourist exchange).

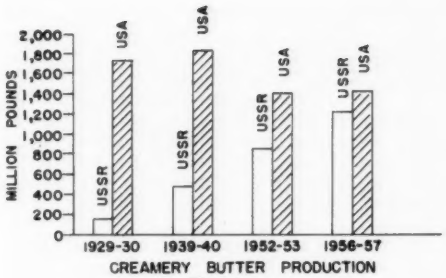


FIG. 4.

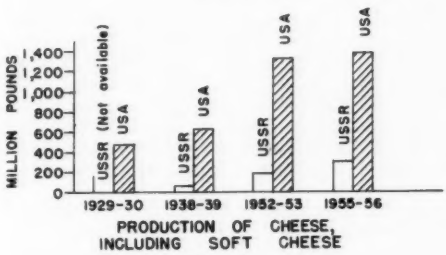


FIG. 5.

The greatest single deterrent in Soviet agriculture is the fact that they have not yet found a way to produce the crops necessary to sustain a large animal agriculture. True, the Soviets grow more than twice as much wheat as does the United States. They grow more oats, more barley, and 25 times as much rye; but this year they produced only about 1/12 the amount of corn grown in the United States. Most of the corn is used in silage and since their seasons are short, it is difficult to mature it as grain. Considerable effort is being expended to develop new varieties for shorter growing seasons.

*Mechanization.* The Soviets are highly mechanized when it comes to tractor power, plowing, tilling, planting, and combining grains; and it is true that their agriculture is still primarily a grain economy. However, in many of their other farm operations they are poorly mechanized. A great deal of human labor is still employed, particularly women. The U.S.S.R. now has about one tractor for every 350 to 400 acres of arable land, compared to one for every 80 to 100 acres in the United States.

There is increased effort in farmstead mechanization but, as a measure of their status in the dairy industry, about 88% of all the cows in Russia are still milked by hand—mostly by women. There are virtually no farm fences. Nearly all livestock except those in barns or barnyards are shepherded.

For many years, much of the Soviet equipment has been copied from the United States and Europe; but there is evidence that they are making their equipment based on innovations of their own. For example, an experimental model of a large power take-off manure spreader was seen. It was equipped with hydraulic motors and with a movable, reversible bed so that the spreader could be tilted back, pushed into a pile of manure, and loaded with the same mechanization as used to spread the material in the field when reversed.

Most Russians seemed to be adequately fed but often poorly clothed. They have conquered hunger and famine, but when it comes to efficiency and mechanization on the farm, there is no question but that they are far behind the United States. In any measure, the U.S.A. produces more than the Soviets by an average of three to six times in terms of man-hours.

Veterinarians play an important part in the dairy industry in Russia, just as they do in the United States. The usual milk inspection procedures such as butterfat testing, sediment checking, bacterial culture, etc. are conducted with milk brought to the market. This is not always done by veterinarians, but is usually under their supervision.

At none of the farms visited was milk being pasteurized. Any cooling of milk on farms is primarily by cold water or ice, although the majority of milk is not cooled at all on farms. It is shipped to a receiving station where it is pasteurized or processed.

### DAIRY PROCESSING

The amount of mechanization of dairy processing plants can be judged by the use of 6,300 pasteurizers, 50,000 separators, and 3,000 refrigeration compressors in the Soviet Union in 1957 (20). The consumption of dairy products in the Soviet Union is about 750 qt. of milk, 8 lb. of butter, and 8 lb. of margarine per person annually.

All milk sold in bottles in Moscow and in other major cities is pasteurized. Milk in markets and in the streets in milk trucks was measured out or drawn off in buckets and bottles for people as they passed by. The technicians at the Moscow Milk Combine told us that usually this milk is not pasteurized, although some of it might be.

Some of the milk had a slight scorched taste. Milk brought to the plant must be 3.2% butterfat or above, but no extra payment is made for higher butterfat content. It is interesting, however, that contrary to practice in the United States, farmers are paid a premium for extra milk they produce. This indicates that it is definitely a government policy to sponsor increased milk production in the U.S.S.R.

Milk moved from the farm to collecting or receiving stations to the processing plant. In the Moscow area the collecting station paid an average base price of 1 ruble 5 kopeks per liter for milk received from the farms. At

Zaporozhe, where milk was hauled 45 kilometers without being cooled, the price was 1 ruble 10 kopeks per liter. For milk produced above an established quota an additional 60 kopeks per liter was paid above the base price. The fluid milk processing plants pay the receiving stations 1 ruble 5 kopeks per liter (1,120 rubles per 2,200 lb.). Milk delivered to manufacturing plants for canning and for cheese received a slightly higher price—about 10 kopeks per liter more than fluid milk.

At Kiev, the price of milk in stores was 2 rubles and 50 kopeks for 0.5 liter. For homogenized milk, the price was 2 rubles and 70 kopeks for 0.5 liter. An extra charge of 5 kopeks per bottle was made for home delivery. A can of condensed milk sold for 5 rubles; whipped butter, 17.5 rubles per kilo; regular butter, 28.5 rubles per kilo; cheese, 29 rubles per kilo; cottage cheese, 13 rubles per kilo. In Moscow, comparable prices were found. The increase in butter and cheese production is shown in Figures 4 and 5.

Pasteurization is done at 74° C. (167° F.) with a 20-sec. plate holder. Standardization of milk is not practiced. Brine cooling is used in the plants. Pasteurized milk is delivered in bulk to factory centers and working centers. Bottled milk production requires 3.6 man-hours per ton and the lowest that has been obtained in a modern plant visited in Moscow was 3.43 man-hours per ton for the month of July for the production of pasteurized bottled milk, most of which was in liter and half-liter containers.

There are five milk enterprises in Moscow, of which the one visited was the newest and still under construction. The total plant occupies four floors of 16,000 sq. m per floor or 64,000 sq. m (about 576,000 sq. ft.) An apartment house is being built as part of the factory which will have 10,000 sq. m of living space for the workers. The plant has been in operation for 3 yr. The plant processes about 500 tons of milk per 24 hr. and most of the fluid milk is placed in one-half-liter bottles. A refrigeration capacity of 1,200,000 calories per hour (400 ton) was used. Four 24-head vacuum filling machines, each with a capacity of 12,000 glass bottles per hour, were being used. Automatic case fillers placed 20 bottles in a case. In addition to bottled milk they make cream with 10% butterfat, fresh cheese, cottage cheese, cream cheese with raisins, and kafir milk.

An Alpha-Laval Swedish pasteurizing unit was used, with plate holding section. The apparatus pasteurized 10 tons per hour. Some tinned-copper lines and some brass valves which were plated were in use. The storage tanks were painted green on the inside and white on the outside. A stand pipe glass in the front of each tank, similar to a boiler water level tube, was used to indicate the level of milk in the tank. Thirty tanks (about 2,000 gal.) were in the plant. A three-blade agitator which operates at 65 r.p.m. was used for mixing. A spray cleaning apparatus consisting of a pipe approximately 4 ft. long, which extended down from the top of the tank, with a 3-in. diameter perforated ball at the end, was installed in each tank. The cleaning solution was forced in at 30 to 40 p.s.i.g. for spraying the tank. All of the milk was clarified in Alpha-Laval units as received.

The cheese packaging line was well-mechanized. The cheese was pulverized and handled mechanically and put in wax paper or paraffin. The small cheese carried the label, Cpipok. For packaging, the product was placed in a cell over a parchment and a plunger from above forced the product into the parchment in the cell. A well-equipped laboratory was available and the control tests consisted of a general count of bacteria, the quality of the product as affected by bacteria, sediment counts, and a check for heat-resistant organisms.

When the production plan is met, the pay is increased 20% over the base price. For each 1% over the plan, the employee receives from 1 to 3%, depending upon his salary.

With milk, just as with wheat, the Russians found it almost impossible to believe that in the U.S.A. there is a surplus of butterfat and other animal fats, and that people were paid less to produce milk used in manufacturing than for fluid milk. They were also amazed to learn that efforts were being made in the United States to develop animals that would produce a higher percentage of solids other than fat and to produce meat animals with less fat. Fat has always been a short item in the Soviets. It is for this reason that they have greatly expanded their production of sunflowers and castor beans.

The dairy industry in the U.S.S.R. gets top priority from the Kremlin leaders, as does agriculture in general. Yet, both in quantitative production and quality of most dairy products, Russia is far behind the United States.

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# OUR ASSOCIATION

## *Program*

## FIFTY-FIFTH ANNUAL MEETING
OF THE

# AMERICAN DAIRY SCIENCE ASSOCIATION

**UTAH STATE UNIVERSITY**
**LOGAN**

**JUNE 19–22, 1960**

PROGRAM COMMITTEE

N. L. JACOBSON, Iowa, Chairman
K. L. TURK, New York, Ex Officio
A. J. MORRIS, Utah
G. W. TRIMBERGER, New York
STUART PATTON, Pennsylvania
C. W. NIBLER, Nebraska

## GENERAL PROGRAM

**Friday, June 17**

9:00 A.M. **Meeting of Executive Board Committees,** Student Union Building

**Saturday, June 18**

9:00 A.M. **Meeting of Executive Board,** Student Union Building

**Sunday, June 19**

9:00 A.M.–12:00 M.
**Meeting of Executive Board,** Student Union Building

10:00 A.M.–10:00 P.M.
**Registration,** Student Union Building

8:00 P.M. **Informal Get-Together,** Student Union Building

**Monday, June 20**

5:30 P.M. **Chicken Barbeque,** Amphitheater

8:00 P.M. **L. D. S. Tabernacle Choir,** Field House
LESTER HEWLETT, President

**Tuesday, June 21**

10:15 A.M.–12:15 P.M.

**General Business Meeting of the Association,** A. C. FAY, Chairman, University Auditorium, Old Main

**Comments of Welcome,** DR. DARYL CHASE, President of Utah State University

4:30 P.M. **Past Presidents' Dinner**

8:00 P.M. **Recognition Program and Presentation of Awards,** University Auditorium

**Wednesday, June 22**

1:00 P.M.–4:00 P.M. Field House

**Address: The Honorable Ezra Taft Benson, Secretary of Agriculture**

**Symposium: Facts, Fallacies, and Conflicts Affecting the Demand for Dairy Foods**

**Moderator:** W. D. KNOX, Editor, Hoard's Dairyman

# MANUFACTURING SECTION

**Monday, June 20**

8:15–11:00 A.M.

ALL SECTIONS. **Milk Lipids Symposium**
Edith Bowen Auditorium

11:15 A.M.–12:00 M.

MANUFACTURING SECTION BUSINESS MEETING
Edith Bowen Auditorium

1:30– 4:00 P.M.

SECTION A. **Dairy Chemistry**
Edith Bowen Auditorium

SECTION B. **Dairy Manufacturing Extension**
Edith Bowen, Room 9

SECTION C. **Antibiotics and Market Milk**
Rural Arts Building

4:00– 5:00 P.M.

MANUFACTURING SECTION BUSINESS MEETING
Edith Bowen Auditorium

5:00– 6:00 P.M.

PRODUCTS JUDGING COMMITTEE AND COACHES' MEETING
Edith Bowen, Room 9

## Tuesday, June 21

8:30–10:00 A.M.

SECTION A. **Dried Products**
Edith Bowen Auditorium

SECTION B. **Cottage Cheese**
Edith Bowen, Room 9

SECTION C. **Concentrated and Frozen Products**
Rural Arts Building

9:00–10:00 A.M.

PROGRAM SPONSORED BY ASSOCIATION'S EDUCATION COMMITTEE
Forestry Building, Room 305

10:15 A.M.–12:15 P.M.

GENERAL BUSINESS MEETING OF THE ASSOCIATION
University Auditorium, Old Main

1:30– 4:30 P.M.

SECTION A. **Dairy Chemistry**
Edith Bowen Auditorium

SECTION B. **Dairy Manufacturing Extension**
Edith Bowen, Room 9

SECTION C. **Dairy Bacteriology**
Rural Arts Building

2:00– 4:30 P.M.

**Symposium: Milk Solids-Not-Fat** (See Production Section)
Forestry Building Auditorium

## Wednesday, June 22

8:30–11:30 A.M.

SECTION A. **Milk Proteins**
Edith Bowen Auditorium

SECTION B. **Market Milk**
Edith Bowen, Room 9

SECTION C. **Butter and Cheese**
Rural Arts Building

1:00– 4:00 P.M.

JOINT WITH PRODUCTION AND EXTENSION SECTIONS
Field House

**An Address: The Honorable Ezra Taft Benson, Secretary of Agriculture**

**Symposium: Facts, Fallacies, and Conflicts Affecting the Demand for Dairy Foods.**

**Moderator:** W. D. KNOX, Hoard's Dairyman, Fort Atkinson, Wisconsin

# PRODUCTION SECTION

## Monday, June 20

8:00–11:00 A.M.

SECTION A. **Reproductive Physiology**
Forestry Building Auditorium

SECTION B. **Forage and Pasture**
Forestry Building, Room 230

11:00 A.M.–12:00 M.

**Production Section Business Meeting**
Forestry Building Auditorium

1:00– 5:00 P.M.

SECTION A.
Forestry Building Auditorium
1:00–4:00 P.M. **Nutrition and Management**
4:00–5:00 P.M. **Joint with Extension Section**
**Reducing Milk Production Costs**
Forestry Building Auditorium

5:00– 6:00 P.M.

**Joint with Extension Section**
**Joint Committee Reports**
**Dairy Cattle Breeding**
**Dairy Cattle Type**
**Dairy Cattle Health**
Forestry Building Auditorium

1:00–5:00 P.M.

SECTION B. **Rumen Physiology**
Forestry Building, Room 230

## Tuesday, June 21

8:00–10:00 A.M.

SECTION A. **Genetics**
Forestry Building Auditorium

SECTION B. **Milk Secretion**
Forestry Building, Room 230

9:00–10:00 A.M.

PROGRAM SPONSORED BY ASSOCIATION'S EDUCATION COMMITTEE
Forestry Building, Room 305

10:15 A.M.–12:15 P.M.

GENERAL BUSINESS MEETING OF THE ASSOCIATION
University Auditorium, Old Main

2:00– 4:30 P.M.

SECTION A. **Joint with Extension Section**

**Symposium: Milk Solids-Not-Fat**
Forestry Building Auditorium

SECTION B. **Reproductive Physiology**
Forestry Building, Room 230

4:30– 5:30 P.M.

JOINT WITH EXTENSION SECTION

**Meeting of the Superintendents of Official Testing**
Forestry Building Auditorium

**Wednesday, June 22**

8:00 A.M.–12:00 M.

SECTION A. **General Physiology**
Forestry Building Auditorium

SECTION B. **Nutrition**
Forestry Building, Room 230

1:00– 4:00 P.M.

JOINT WITH MANUFACTURING AND EXTENSION SECTIONS
Field House

**An Address: The Honorable Ezra Taft Benson, Secretary of Agriculture**

**Symposium: Facts, Fallacies, and Conflicts Affecting the Demand for Dairy Foods**

**Moderator:** W. D. KNOX, Hoard's Dairyman, Fort Atkinson, Wisconsin

# EXTENSION SECTION

**Monday, June 20**

8:00–10:30 A.M.

**Dairy Records**
Ag. Science, Room 205

10:30 A.M.–12:00 M.

EXTENSION SECTION BUSINESS MEETING
Ag. Science, Room 205

1:00– 3:50 P.M.

**Teaching Methods**
Ag. Science, Room 205

4:00– 6:00 P.M.

JOINT WITH PRODUCTION SECTION

**Joint Committee Reports**
Forestry Building Auditorium

**Tuesday, June 21**

8:10–10:00 A.M.

**4-H Program**
Ag. Science, Room 205

10:15 A.M.–12:15 P.M.

GENERAL BUSINESS MEETING OF ASSOCIATION
University Auditorium, Old Main

2:00– 4:30 P.M.

JOINT WITH PRODUCTION SECTION

**Symposium: Milk Solids-Not-Fat**
Forestry Building Auditorium

4:30– 5:30 P.M.

JOINT WITH PRODUCTION SECTION

**Meeting of Superintendents of Official Testing**
Forestry Building Auditorium

**Wednesday, June 22**

8:00–10:00 A.M.

**A.B. Proved Sires and Mastitis Control**
Ag. Science, Room 205

10:00 A.M.–12:00 M.

**The Evaluation of Forages**
Ag. Science, Room 205

1:00– 4:00 P.M.

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS
Field House

**An Address: The Honorable Ezra Taft Benson, Secretary of Agriculture**

**Symposium: Facts, Fallacies, and Conflicts Affecting the Demand for Dairy Foods**

**Moderator:** W. D. KNOX, Hoard's Dairyman, Fort Atkinson, Wisconsin

# STUDENT BRANCH SECTION

**Monday, June 20**

9:00 A.M.

**Registration**
Student Union, Room 226

10:00 A.M.

**Organization Meeting and Committee Appointments**
Student Union, Room 226

11:30 A.M.
**Special Luncheon with Certain A.D.S.A. Members**

**Tuesday, June 21**

8:30 A.M.
**Committee Reports and Discussions**
Student Union, Room 226

**Wednesday, June 22**

8:30 A.M.
**Discussion of Exhibits, Election, and Meeting of Officers with Student Branch Committees**
Student Union, Room 226

# ENTERTAINMENT

## GENERAL PROGRAM

| | |
|---|---|
| Sunday, June 19 | 8:00 P.M.—**Informal Get-Together**<br>Student Union Building |
| Monday, June 20 | 5:30 P.M.—**Chicken Barbeque**<br>Amphitheater |
| | 8:00 P.M.—**L.D.S. Tabernacle Choir**<br>Field House |
| Tuesday, June 21 | 8:00 P.M.—**Recognition Program and Presentation of Awards**<br>University Auditorium, Old Main |

## WOMEN'S PROGRAM

| | |
|---|---|
| Monday, June 20 | 9:00 A.M. to 11:30 A.M.—**Visit University Campus and Shopping in Logan Business District** |
| | 1:30 P.M.—**Tea at the Home of President Chase,** Campus |
| | 5:30 P.M.—**Chicken Barbeque**<br>Amphitheater |
| | 8:00 P.M.—**L.D.S. Tabernacle Choir**<br>Field House |
| Tuesday, June 21 | 10:15 A.M. to 12:15 P.M.—**General Business Meeting of the Association**<br>University Auditorium, Old Main |

12:15 P.M.—**Special Luncheon**
to 2:00 P.M. Student Union

2:15 P.M.—**Tour at Logan Temple and Tabernacle**
to 4:30 P.M. **Grounds, and Logan Canyon to Point Overlooking Bear Lake.***

3:00 P.M.—**Historic Utah, Story with Pictures.***
to 4:30 P.M. Student Union

8:30 P.M.—**Recognition Program and Presentation of Awards**
University Auditorium

Wednesday, June 22

10:00 A.M.—**Historic Utah, Story with Pictures.**
to 11:00 A.M. (repeat)

## CHILDREN'S PROGRAM

(See Separate Program)

**Children 4 and 5 Years**

Monday, June 20
Tuesday, June 21
Wednesday, June 22

9:30 A.M.—**Supervised Care**
to 11:00 A.M. Nursery School

1:30 P.M.—**Supervised Care**
to 4:30 P.M. Nursery School

**Children 6–12 Years**

Monday, June 20
Tuesday, June 21
Wednesday, June 22

9:30 A.M.—**Recreation on the Quadrangle**
to 11:00 A.M.

1:30 P.M.—**Recreation on the Quadrangle**
to 4:30 P.M.

**Children 8–12 Years**

2:30 P.M.—**Swimming**
to 4:30 P.M. Smart Gymnasium

**Young People—12 and Older**

Monday, June 20

9:00 A.M.—**Tour of Campus and Vicinity**
to 11:00 A.M.

Tuesday, June 21

9:00 A.M.—**To Old Juniper, Logan Canyon**
to 3:00 P.M. Box Lunch

3:00 P.M.—**Swimming**
to 4:30 P.M.

7:30 P.M.—**Teen-age Mixer and Dance**
to 9:30 P.M. Student Union Ball Room

* Optional tour or historic pictures.

Wednesday, June 22

9:30 A.M.—**Recreation on the Quadrangle**
to 11:30 A.M.

1:30 P.M.—**Recreation on the Quadrangle**
to 4:00 P.M.

2:30 P.M.—**Swimming**
to 4:30 P.M. Smart Gymnasium

# MANUFACTURING SECTION

**Monday, June 20**

8:15–11:00 A.M.

ALL SECTIONS. **Milk Lipids Symposium**

STUART PATTON, Chairman
Edith Bowen Auditorium

Milk fat synthesis. J. R. LUICK, University of California.

Gas chromatographic analysis. STUART PATTON, Pennsylvania State University.

Origin of depot fat. S. B. TOVE, North Carolina State College.

Autoxidation of milk fat. E. A. DAY, Oregon State College.

11:15–12:00 M.

MANUFACTURING SECTION BUSINESS MEETING
STUART PATTON, Chairman
Edith Bowen Auditorium

1:30– 4:00 P.M.

SECTION A. **Dairy chemistry**

E. L. JACK, Chairman
Edith Bowen Auditorium

M1. The quantitative determination of fatty acids by gas chromatography. C. W. GEHRKE, D. F. GOERLITZ, C. O. RICHARDSON, AND H. D. JOHNSON, University of Missouri.

M2. Application of gas chromatography to the study of volatiles formed in milk fat upon heating. W. W. NAWAR, F. M. SAWYER, W. FLETCHER, AND I. S. FAGERSON, University of Massachusetts.

M3. The crystallization behavior of milk fat. J. M. DEMAN, University of Alberta, Edmonton, Canada.

M4. The trisaturated glycerides of butteroil. E. G. HAMMOND, Iowa State University.

M5. Distearyl triglycerides in the high melting fraction of milk fat. P. G. KEENEY, Pennsylvania State University.

M6. The fatty acid composition of the monoglycerides from lipolyzed milk fat. R. G. JENSEN AND G. W. GANDER, University of Connecticut.

M7. The characterization of the oxidized flavor in butteroil. A. M. EL-NEGOUMY AND E. G. HAMMOND, Iowa State University.

M8. The relationship of sensory to chemical methods for measuring the oxidized flavor of milk fat. D. A. LILLARD AND E. A. DAY, Oregon State College.

M9. Studies on rapid determination of lactose in raw milk. N. W. DURRANT AND K. W. STONE, Virginia Polytechnic Institute.

M10. The kinetics of milk acid phosphatase. G. C. KRESHECK, W. J. HARPER, AND T. KRISTOFFERSEN, Ohio State University.

1:30– 4:00 P.M.

SECTION B. **Dairy Manufacturing Extension**

R. B. REDFERN, Chairman
Edith Bowen, Room 9

TECHNOLOGICAL ASPECTS OF DAIRY PRODUCTS MANUFACTURING:

Advertising and merchandising research in the dairy industry. JACK WEBER, University of Idaho.

Trends in dairy products merchandising and their impact on the dairy industry. EDWARD THOM, The Olsen Publishing Co., Milwaukee, Wisconsin.

Modern concepts of dairy plant engineering. A. L. RIPPEN, Michigan State University

Trends in ultra-high temperature pasteurization of dairy products. W. M. ROBERTS, North Carolina State College.

1:30– 4:00 P.M.

SECTION C. **Antibiotics and Market Milk**

BURDET HEINEMANN, Chairman
Rural Arts Building

M11. The milk and microbial lipases as affected by antibiotics. R. C. CHANDON AND K. M. SHAHANI, University of Nebraska.

M12. Rapid antibiotic assay methods using *Bacillus stearothermophilus*. R. T. IGARASHI, R. W. BAUGHMAN, F. E. NELSON, AND P. A. HARTMAN, Iowa State University.

M13. A study of dye reduction methods as platform tests for the detection of antibiotics. BURDET HEINEMANN, Producers Creamery Co., Springfield, Missouri.

M14. Problems of design in simple field tests for antibiotics in milk. F. V. KOSIKOWSKI AND R. A. LEDFORD, Cornell University.

M15. Penicillin levels in milk following parenteral administration of procaine penicillin G. YUSAF VAID, C. C. PROUTY, A. O. SHAW, AND R. E. WATTS, Washington State University.

M16. The use of antifungal agents in the retardation of Cottage Cheese spoilage. K. M. NILSON, M. CARRANCEDO, AND K. M. SHAHANI, University of Nebraska.

M17. Further studies of lipase activity in the milk of individual cows. E. S. GUTHRIE AND B. L. HERRINGTON, Cornell University.

M18. The influence of storage temperature on lipolytic activity in individual cows' milk. P. E. JOHNSON AND R. L. VON GUNTEN, Oklahoma State University.

M19. Relation between the phosphatase activity of raw and pasteurized milk. J. B. MICKLE, Oklahoma State University.

M20. The effect of temperature on the keeping qualities of milk in market channels. G. E. HUSKEY, J. E. EDMONDSON, AND K. L. SMITH, University of Missouri.

4:00– 5:00 P.M.

MANUFACTURING SECTION BUSINESS MEETING
STUART PATTON, Chairman
Edith Bowen Auditorium

5:00– 6:00 P.M.

PRODUCTS JUDGING COMMITTEE AND COACHES' MEETING
E. L. THOMAS, Chairman
Edith Bowen, Room 9

## Tuesday, June 21

8:30–10:00 A.M.

SECTION **A. Dried Products**
STUART PATTON, Chairman
Edith Bowen Auditorium

M21. Manufacture of high-acid powder from Cottage Cheese whey and skimmilk. J. C. BARNES, C. M. STINE, AND T. I. HEDRICK, Michigan State University.

M22. Comparison of flavor and some other chemical properties of noninstantized and instantized nonfat dry milk. J. B. MANN AND J. M. MCINTIRE, Carnation Company, Van Nuys, California.

M23. The effect of an oxygen scavenger "packet," desiccant in-package system on the stability of dry whole milk and dry ice cream mix. R. I. MEYER AND LOUIS JOKAY, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois.

M24. The effect of forewarming treatments on the flavor of nonfat dry milk. MAXINE MCDIVITT, A. M. SWANSON, AND LA VELLE WITTWER, University of Wisconsin.

M25. Effect of moisture content, storage temperature, and oxygen level on flavor stability of foam-dried whole milk. ARJEN TAMSMA, M. J. PALLANSCH, T. J. MUCHA, AND W. I. PATTERSON, Eastern Utilization Research and Development Division, USDA, Washington, D.C.

M26. Reconstitution of dry milk products. G. H. PYNE AND S. T. COULTER, University of Minnesota.

8:30–10:00 A.M.

SECTION B. **Cottage Cheese**
M. L. SPECK, Chairman
Edith Bowen, Room 9

M27. The influence of package upon the temperature of Cottage Cheese in retail marketing channels. J. W. SHERBON AND E. L. THOMAS, University of Minnesota.

M28. A study of the surface spoilage defect of Cottage Cheese. H. K. ZIMMERMAN AND L. T. KESTER, Carnation Company, Van Nuys, California.

M29. The use of frozen culture as a direct inoculum for making Cottage Cheese. A. W. RUDNICK, JR. AND W. E. GLENN, University of Kentucky.

M30. Factors affecting the total lactic acid concentration in Cottage Cheese. H. RANDOLPH AND W. J. HARPER, Ohio State University.

M31. A reduction test to predict the keeping quality of Cottage Cheese. L. G. HARMON AND G. C. WALKER, Michigan State University.

M32. The keeping quality of Cottage Cheese packaged under high vacuum, nitrogen, and carbon dioxide. C. N. TSANTILIS AND F. V. KOSIKOWSKI, Cornell University.

8:30–10:00 A.M.

SECTION C. **Concentrated and Frozen Products**

BURDET HEINEMANN, Chairman

Rural Arts Building

M33. The reactions of consumers to sterile concentrated milk. H. E. CALBERT AND H. C. JACKSON, University of Wisconsin.

M34. A study of some factors related to the storage life of sterilized concentrated milk. M. E. SEEHAFER AND A. M. SWANSON, University of Wisconsin.

M35. The effect of deaeration on the flavor stability of concentrated sweetened cream. H. A. ANDERSON, R. W. BELL, AND R. P. TITTSLER, Eastern Utilization Research and Development Division, USDA, Washington D.C.

M36. Resistance to heat shock damage in ice cream containing corn sweeteners with different dextrose equivalents. P. G. KEENEY AND D. V. JOSEPHSON, Pennsylvania State University.

M37. Variations of soft-serve ice cream churning times caused by certain rations fed to dairy cows. D. R. FRAZEUR AND C. H. NOLLER, Purdue University.

M38. An explanation for reduced incidence of sandiness. T. A. NICKERSON, University of California.

9:00–10:00 A.M.

PROGRAM SPONSORED BY ASSOCIATION'S EDUCATION COMMITTEE

Forestry Building, Room 305

10:15 A.M.–12:15 P.M.

GENERAL BUSINESS MEETING OF THE ASSOCIATION

A. C. FAY, Chairman

University Auditorium, Old Main

1:30– 4:30 P.M.

SECTION A. **Dairy Chemistry**

STUART PATTON, Chairman

Edith Bowen Auditorium

M39. Effects of surface-active agents on milk lipase activity. V. S. PACKARD AND J. J. JEZESKI, University of Minnesota.

M40. Some factors influencing the A-, B-, and C-esterase activity of cow's milk. T. L. FORSTER, M. W. MONTGOMERY, AND J. E. MONTOURE, Washington State College.

M41. The specificity of milk lipase toward the primary ester groups of triglycerides. G. W. GANDER AND R. G. JENSEN, University of Connecticut.

M42. Comparison of the Cenco, Ohaus, and Mojonnier methods for determining total solids of dairy products. M. K. HOEFLIN AND J. J. SHEURING, University of Georgia.

M43. Exploratory analyses of silage volatiles which may affect milk flavor. M. E. MORGAN, University of Connecticut.

M44. Gas chromatography of volatiles of fermented milk products. V. S. BAVISOTTO, L. A. ROCK, AND R. S. LESNIEWSKI, Paul-Lewis Laboratories, Milwaukee, Wisconsin.

M45. Volumetric determination of calcium and magnesium in milk and blood plasma. T. H. KAMAL, University of Missouri.

M46. Heat coagulation of milk from individual cows. JOHN BELEC AND ROBERT JENNESS, University of Minnesota.

M47. Sedimentation of reconstituted concentrated milks. H. K. WILSON AND E. O. HERREID, University of Illinois.

M48. Ferricyanide reduction values of different milks. I. I. PETERS AND C. A. MULAY, Texas Agricultural and Mechanical College.

M49. The effect of the structure of some mercaptans on their amperometric titration with silver nitrate. K. AIBARA AND E. O. HERREID, University of Illinois.

M50. Effect of storage, preheat treatment, concentration, and sterilization on the heat-produced sulfhydryl compounds of milk. P. S. RAO AND I. A. GOULD, Ohio State University.

1:30– 4:30 P.M.

SECTION B. **Dairy Manufacturing Extension**

W. S. ARBUCKLE, Chairman

Edith Bowen, Room 9

TECHNIQUES USED IN DAIRY TECHNOLOGY EXTENSION

The role of the dairy technologist in extension. C. M. FERGUSON, Federal Extension Service, USDA, Washington D.C.

The new dairy industry plant training manual and its application by dairy technologists. H. B. HENDERSON, University of Georgia.

PANEL

A management improvement program. D. J. HANKINSON, University of Massachusetts.

Co-ordinated state-wide programs for producers' milk quality improvement. EVERT WALLENFELDT, University of Wisconsin.

The role of short courses, conferences, and newsletters in personnel training. M. E. GREGORY, Ohio State University.

Survey analysis of problems in dairy products manufacturing. C. B. REEVES, Clemson Agricultural and Mechanical College.

Working with industry organizations. R. B. REDFERN, North Carolina State College.

Summary. W. S. ARBUCKLE.

DAIRY MANUFACTURING EXTENSION BUSINESS MEETING

1:30– 4:30 P.M.

SECTION C. **Dairy Bacteriology**

M. L. SPECK, Chairman

Rural Arts Building

M51. Thermoduric microorganisms present in bulk-tank farm milk in Iowa. R. A. BUCHANAN AND F. E. NELSON, Iowa State University.

M52. Substituting the membrane filter for the standard plate count in the bacteriological examination of milk. R. T. MARSHALL AND J. E. EDMONDSON, University of Missouri.

M53. Anaerobic studies with antibiotic-sensitive bacteria. R. A. LEDFORD AND F. V. KOSIKOWSKI, Cornell University.

M54. Effect of pH and brine concentration on the thermal process required to preserve canned cheese spreads using PA 3678 as a test organism. J. A. JAYNES, I. J. PFLUG, AND L. G. HARMON, Michigan State University.

M55. Chemical changes in bacteria heated in milk and other media. W. A. MOATS, Market Quality Research Division, USDA, Beltsville, Maryland.

M56. Growth of staphylococci in condensed skimmilk at low and moderate temperatures. E. GEORGE, JR. AND J. C. OLSON, JR., University of Minnesota.

M57. Bactericidal effectiveness of hydrogen-peroxide-catalase treatments on *Staphylococcus aureus*. N. F. OLSON AND W. V. PRICE, University of Wisconsin.

M58. Galactokinase and galactowaldenase enzyme activity of *Streptococcus lactis*. K. M. SHAHANI, University of Nebraska.

M59. Antibiotic resistance of starter cultures belonging to the streptococci. R. J. RICHARDS AND H. E. KENNEDY, Ohio State University.

M60. Acid production in the presence of antibiotics. I. Oxytetracycline inactivating properties of a resistant strain of *Streptococcus lactis*. E. MIKOLAJCIK, W. J. HARPER, AND H. E. KENNEDY, Ohio State University.

M61. Acid production in the presence of antibiotics. II. Effect of nutritional additives on oxytetracycline- or penicillin-resistant cultures. H. E. KENNEDY, Ohio State University.

M62. The heat resistance of four strains of psychrophilic organisms added to skimmilk for Cottage Cheese manufacture. R. A. CHAUDHARY, S. L. TUCKEY, AND L. D. WITTER, University of Illinois.

2:00– 4:30 P.M.

**Symposium: Milk Solids-Not-Fat** (See Production Section)
Forestry Auditorium

**Wednesday, June 22**

8:30–11:30 A.M.

SECTION A. **Milk Proteins**
C. A. ZITTLE, Chairman
Edith Bowen Auditorium

M63. Characteristics of the "minor" proteins of milk. M. P. THOMPSON AND J. R. BRUNNER, Michigan State University.

M64. Isolation and characterization of a membrane-protein fraction from the fat-globule membrane of raw milk. K. S. RAMACHANDRAN AND R. McL. WHITNEY, University of Illinois.

M65. Denaturation of the serum proteins of skimmilk heated by direct steam injection. C. W. DILL, W. M. ROBERTS, M. W. CARTER, AND H. L. LUCAS, North Carolina State College.

M66. Estimation of the protein content of milk by dye binding with Buffalo Black. C. VANDERZANT AND W. R. TENNISON, Texas Agricultural and Mechanical College.

M67. Total milk protein and serum protein by steam distillation. W. K. STONE, L. F. CREMERS, AND N. R. THOMPSON, Virginia Polytechnic Institute.

M68. Column chromatography of casein on the adsorbent diethylaminoethyl (DEAE)-cellulose. C. A. ZITTLE, Eastern Utilization Research and Development Division, USDA, Philadelphia, Pennsylvania.

M69. Molecular structural studies on casein. W. G. GODBEY AND C. W. GEHRKE, University of Missouri.

M70. A method for the isolation of the calcium-insensitive casein fraction from whole casein. H. E. SWAISGOOD AND J. R. BRUNNER, Michigan State University.

M71. The effect of pH and concentration of soluble calcium on rennet curd tension. U. S. ASHWORTH AND JOSEPH NEBE, Washington State University.

M72. Electrophoretic changes in casein and casein fractions resulting from rennin. J. O. YOUNG AND U. S. ASHWORTH, Washington State University.

M73. Spectrophotometric observations on the reaction of rennin with sodium caseinate. L. M. LIBBEY AND U. S. ASHWORTH, Washington State University.

M74. A study of the nonprotein nitrogen liberated by the action of rennin on casein. I. S. VERMA AND F. H. GRIMBLEBY, University of Reading, Reading, England.

8:30–11:30 A.M.

SECTION B. **Market Milk**

BURDET HEINEMANN, Chairman

Edith Bowen, Room 9

M75. Evaluation of circulation cleaning of welded pipelines. R. B. MAXCY AND K. M. SHAHANI, University of Nebraska.

M76. The use of $P^{32}$ in the study of the cleanability of milk-handling surface. J. J. PETERS, The Nestlé Company, Marysville, Ohio, and H. E. CALBERT, University of Wisconsin.

M77. The incidence of potentially pathogenic staphylococci in dairy products at the consumer level. V. D. FOLTZ, R. MICKELSEN, AND W. H. MARTIN, Kansas State University, and C. A. HUNTER, Kansas State Board of Health, Topeka.

M78. Removal of $CS^{134}$ and $K^{42}$ from milk with ion exchange resins. B. J. DEMOTT AND D. G. EASTERLY, University of Tennessee.

M79. Studies on the stability of frozen milk. I. D. DESAI, T. A. NICKERSON, AND W. G. JENNINGS, University of California.

M80. The use of Gerber reagents with Babcock glassware and equipment as a test for fat in homogenized and unhomogenized chocolate milk. J. T. CARDWELL, Mississippi State University.

M81. The density plastic sphere hydrometer for determining solids-not-fat on bi-monthly composite samples of producers' herd milk. N. S. GOLDING AND L. J. MANUS, Washington State University.

M82. Methods of payment for producer farmers' milk used for a retail market on its actual weight delivered and composition (per cent fat and per cent S.N.F.) as obtained from bi-monthly composite samples for one year. L. J. MANUS AND N. S. GOLDING, Washington State University.

M83. Effectiveness of various vacuum, temperature, and steam treatments in reducing feed flavors in milk. E. C. COTNER, W. H. MARTIN, R. MICKELSEN, AND W. D. RUTZ, Kansas State University.

M84. Effect of single-chamber vacuum[1] treatment on flavor and losses of milk. ALEC BRADFIELD, University of Vermont.

M85. Influence of oat and alfalfa hay on susceptibility of milk to oxidized flavor. W. L. DUNKLEY, L. M. SMITH, AND M. RONNING, University of California.

M86. A technique for studying the effects of feed and environment on the flavor of milk. R. W. DOUGHERTY AND W. F. SHIPE, Cornell University.

8:30–11:30 A.M.

SECTION C. **Butter and Cheese**

M. L. SPECK, Chairman

Rural Arts Building

[1] Vacu-therm is name designated by the manufacturing company.

M87. The effect of antioxidants on the development of oxidized flavors in cold storage butter. A. M. EL-NEGOUMY AND E. G. HAMMOND, Iowa State University.

M88. The influence of butterfat precrystallization on the hardness of continuously made butter. F. W. WOOD AND L. M. MCKNIGHT, University of Alberta, Edmonton, Canada.

M89. Further studies of the effect of edible additives on the spreading quality of butter. J. G. KAPSALIS AND T. KRISTOFFERSEN, Ohio State University.

M90. A study of the effect of added mono- and di-glycerides on the body and texture of cream and Neufchatel cheeses. MAX MILLER, C. M. STINE, AND L. G. HARMON, Michigan State University.

M91. Quantitative estimation of carbonyl compounds in Limburger cheese. JOHN COLMEY AND S. L. TUCKEY, University of Illinois.

M92. Retention of moisture by Blue Cheese. L. A. RICHARDSON, H. A. MORRIS, AND W. B. COMBS, University of Minnesota.

M93. Survival of staphylococci in Colby Cheese. G. C. WALKER, L. G. HARMON, AND C. M. STINE, Michigan State University.

M94. A comparison of two end point indicators used in a direct titration method for determining the sodium chloride content of cheese. W. M. BREENE AND W. V. PRICE, University of Wisconsin.

M95. Cheddar Cheese quality as affected by the age of the milk and addition of certain purine compounds. T. KRISTOFFERSEN AND D. D. COLE, Ohio State University.

M96. Effect of different cheddaring methods on the quality of Cheddar Cheese. N. F. OLSON AND W. V. PRICE, University of Wisconsin.

M97. Effect of strain of starter culture and manufacturing procedure on bitterness in Cheddar Cheese. D. B. EMMONS, W. A. MCGUGAN, AND J. A. ELLIOTT, Canada Department of Agriculture, Ottawa, Canada.

M98. Nitrogen distribution in Cheddar Cheese as related to bitterness and strain of starter culture. D. B. EMMONS, W. A. MCGUGAN, AND J. A. ELLIOTT, Canada Department of Agriculture, Ottawa, Canada.

M99. A study of delayed gas formation in Cheddar Cheese. M. E. OLDHAM, University of Wyoming, and A. J. MORRIS, Utah State University.

1:00– 4:00 P.M.

JOINT WITH PRODUCTION AND EXTENSION SECTIONS

Field House

**An Address: The Honorable Ezra Taft Benson, Secretary of Agriculture**

**Symposium: Facts, Fallacies, and Conflicts Affecting the Demand for Dairy Foods**

**Moderator:** W. D. KNOX, Hoard's Dairyman, Fort Atkinson, Wisconsin

Adulterants. M. R. STEPHENS, Food and Drug Administration, Washington, D.C.

Calcium and strontium$^{90}$. MARGARET A. OHLSON, State University of Iowa.

Milk in relation to specific diseases. ZOE E. ANDERSON, Wayne State University.

# PRODUCTION SECTION

**Monday, June 20**

8:00–11:00 A.M.

SECTION A. **Reproductive Physiology**

G. W. TRIMBERGER, Chairman

Forestry Building, Auditorium

P1. The hastening of estrus in cycling virgin female rats by stimulation with air blast. E. W. HAHN AND R. L. HAYS, University of Illinois.

P2. The effect of estradiol on the thyroid secretion rate of dairy cattle. G. W. PIPES, B. N. PREMACHANDRA, AND C. W. TURNER, University of Missouri.

P3. Infertility in dairy cattle due to anestrum. R. M. PORTER AND T. H. BELLING, New Mexico State University.

P4. Some observations on large luteal type ovarian cysts in a herd of dairy cattle. K. R. JOHNSON, University of Idaho.

P5. The use of progesterone-4-$C^{14}$ in the determination of progestins in bovine ovaries and adrenals. F. STORMSHAK AND MARJORIE L. HUNT, Washington State University.

P6. Quantitative levels of progestins in the bovine corpus luteum during pregnancy. R. E. ERB, F. STORMSHAK AND JOAN DIXON, Washington State University.

P7. Estrous cycle length of Jersey and Sindhi-Jersey crossbred cattle in Louisiana. J. L. FLETCHER, Dairy Cattle Research Branch, Agricultural Research Service, Iberia Livestock Experiment Station, Jeanerette, Louisiana.

P8. Mastitis and estrogenic activity. W. D. POUNDEN AND NORMA A. FRANK, Ohio Agricultural Experiment Station, Wooster.

P9. The effect of ovarian hormones on the chemical composition of uterine secretions. R. B. HEAP AND G. E. LAMMING, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, England.

P10. The influence of ovarian hormones on resistance to experimental uterine infections. G. E. LAMMING AND R. B. HEAP, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, England.

P11. Sire effects on reproductive performance of their daughters. E. K. INSKEEP, W. J. TYLER, AND L. E. CASIDA, University of Wisconsin.

P12. Prenatal growth measurements of the bovine. H. T. GIER AND G. B. MARION, Kansas State University.

P13. Diurnal patterns of bovine body temperature. T. R. WRENN, JOEL BITMAN, AND J. F. SYKES, Animal Husbandry Research Division, ARS, Beltsville, Maryland.

P14. Abnormal sex ratios of the young parabiotic rats. E. W. HAHN AND R. L. HAYS, University of Illinois.

8:00–11:00 A.M.

SECTION B. **Forage and Pasture**

S. D. MUSGRAVE, Chairman
Forestry Building, Room 230

P15. The digestibility of grass hays grown under different types of nitrogen application. M. C. STILLIONS, W. V. CHALUPA, J. L. EVANS, AND J. L. CASON, Rutgers—The State University, New Brunswick, New Jersey.

P16. The use of the rabbit in predicting forage digestion by ruminants. C. R. RICHARDS, G. F. W. HAENLEIN, J. D. CONNOLLY, AND M. C. CALHOUN, University of Delaware.

P17. Various levels of citrolas crumbles as an additive to high moisture forage for silage. W. J. MILLER AND H. L. DALTON, University of Georgia.

P18. Consumption and feeding value of silages as affected by dry matter content. C. H. GORDON, J. C. DERBYSHIRE, E. A. KANE, Animal Husbandry Research Division, and D. T. BLACK AND J. R. MCCALMONT, Agricultural Engineering Research Division, ARS, Beltsville, Maryland.

P19. Composition of the oat plant as affected by level of nitrogen fertilization and stage of maturity. O. T. STALLCUP, R. R. ROBERSON, AND R. L. THURMAN, University of Arkansas.

P20. Comparison of silage and Pearl millet for maintaining summer milk production. LOUIS J. BOYD, Tennessee Agricultural Experiment Station, Knoxville.

P21. Supplemental value of dehydrated alfalfa pellets for milking cows fed a high corn silage timothy hay ration. L. D. BROWN, C. A. LASSITER, C. F. HUFFMAN, AND E. J. BENNE, Michigan State University.

P22. The influence of meadow crop silage and soilage and added grain on the efficiency of nitrogen utilization in dairy cows. H. R. CONRAD, J. W. HIBBS, AND A. D. PRATT, Ohio Agricultural Experiment Station, Wooster.

P23. A comparison of first, second, and fifth cuttings of alfalfa for milk production. R. M. PORTER, D. D. MILLER, AND S. R. SKAGGS, New Mexico State University.

P24. Relationship of stage of maturity at harvest to the feeding value of sorghum silage for dairy cows. RAYMOND E. HELM AND R. E. LEIGHTON, Texas Agricultural Experiment Station, College Station.

P25. The apparent digestibility of alfalfa-bromegrass with advancing maturity, using a modified digestion trial. F. A. MARTZ, C. H. NOLLER, AND D. L. HILL, Purdue University.

P26. Nutritional evaluation of pastures with dairy cattle in Louisiana. J. E. BERTRAND, L. L. RUSOFF, J. L. FLETCHER, C. E. HYDE, AND C. BRANTON, Louisiana Agricultural Experiment Station, Baton Rouge, and Iberia Livestock Experiment Station, Jeanerette, Louisiana.

P27. Effect of two levels of grazing intensity upon animal and grazing land productivity. H. J. LARSEN AND G. H. TENPAS, University of Wisconsin.

P28. Harvesting soilage once or twice daily for milking cows. A. D. PRATT, R. R. DAVIS, AND H. R. CONRAD, Ohio Agricultural Experiment Station, Wooster.

11:00 A.M.–12:00 M.

PRODUCTION SECTION BUSINESS MEETING

G. W. TRIMBERGER, Chairman

Forestry Building, Auditorium

1:00 P.M.– 5:00 P.M.

SECTION A.

G. W. TRIMBERGER, Chairman

Forestry Building, Auditorium

1:00– 4:00 P.M.

**Nutrition and Management**

P29. The effect of certain bulk handling practices on the acid degree value of milk. DONALD C. BROWN, B. W. THARP, AND W. R. THOMAS, University of Wyoming.

P30. Studies on immersion cleaning with bucket machine milking plant. R. L. WHITEHOUSE AND L. F. L. CLEGG, University of Alberta, Edmonton, Alberta, Canada.

P31. Feeding values of alfalfa haylage, silage, green-chop, pasture, and artificially dried hay. H. H. VOELKER AND E. BARTLE, South Dakota State College.

P32. Several factors affecting the milk production response of cows to changes in the quantity and nature of the ration. R. E. MATHER, C. P. BREIDENSTEIN, B. R. POULTON, AND G. H. BONNINGTON, JR., New Jersey Agricultural Experiment Station, Sussex.

P33. Milk production and growth of identical twins calving for the first time at two vs. three years of age. E. W. SWANSON, University of Tennessee.

P34. The influence of heat (80° and 90° F.) and stage of lactation at 65° F. on salt balance of milk. T. H. KAMAL, A. C. RAGSDALE, AND H. D. JOHNSON, University of Missouri.

P35. The effect of air conditioning on productive functions of dairy cattle during hot weather. J. E. JOHNSTON, G. A. HINDERY, F. TURNIPSEED, AND D. THOMPSON, Louisiana Agricultural Experiment Station, Baton Rouge.

P36. The effect of constant environmental temperatures and relative humidities on feed digestion by lactating Holstein cows. A. V. DAVIS AND C. P. MERILAN, University of Missouri.

P37. Effects of various combinations of temperature and humidities on milk production. H. D. JOHNSON, H. H. KIBLER, A. C. RAGSDALE, AND M. D. SHANKLIN, University of Missouri and USDA.

P38. Tolerance of dairy cows to high climatic temperatures on low roughage ration. G. H. STOTT, University of Arizona, and E. GRANT MOODY, Arizona State University.

P39. Preliminary observations on the effect of frequency of feeding and equalized intake upon milk production characteristics, body weight, and feed utilization in lactating dairy cows. J. R. CAMPBELL AND C. P. MERILAN, University of Missouri.

P40. Effect of various hay: concentrate ratios and levels of feeding on production and composition of milk of dairy cows. D. K. HOTCHKISS, N. L. JACOBSON, AND C. P. COX, Iowa State University.

P41. Relationship between body weight and yield of dairy cows. R. E. ERB, Washington State University.

P42. Economic analysis of large commercial dairies in Los Angeles County, California. J. L. ALBRIGHT, D. R. PATTON, AND E. A. HYER, University of Illinois; FRAZER AND TORBET, C.P.A., Los Angeles, California State Polytechnic College.

4:00– 5:00 P.M.

JOINT WITH EXTENSION SECTION

G. W. TRIMBERGER, Chairman

Forestry Building, Auditorium

Invitational Paper: Reducing milk production costs. A. SCHULTIS, University of California.

5:00– 6:00 P.M.

JOINT WITH EXTENSION SECTION

**Joint Committee Reports.**

G. W. TRIMBERGER, Chairman

Forestry Building, Auditorium

Dairy Cattle Breeding. C. D. MCGREW, Ohio.

Dairy Cattle Type. E. C. MEYER, Wisconsin.

Dairy Cattle Health. H. P. EWALT, Oregon.

1:00– 5:00 P.M.

SECTION B. **Rumen Physiology**

S. D. MUSGRAVE, Chariman

Forestry Building, Room 230.

1:00– 2:00 P.M.

**Invitational paper:** Continued progress toward controlling bloat. H. H. COLE AND J. M. BODA, University of California.

**Discussant:** H. R. CONRAD, Ohio Agricultural Experiment Station.

2:00– 5:00 P.M.

P43. Observations on the use of plant juices in bloat studies. J. T. BLAKE AND R. S. ALLEN, Iowa State University.

P44. Physiological studies of the rumen with emphasis on the animal factors associated with bloat. V. E. MENDE AND J. M. BODA, University of California.

P45. An antifoaming agent for treatment of pasture bloat. R. H. JOHNSON AND R. S. ALLEN, Iowa State University.

P46. Studies on excessive salivation in ruminants fed certain leguminous forages. J. H. Byers and H. P. Broquist, University of Illinois.

P47. Effect of protein status on salivary urea secretion. Y. Hirose, R. S. Emery, C. F. Huffman, and G. H. Conner, Michigan State University.

P48. The course of cellulose digestion by rumen microorganisms in vitro. R. L. Salsbury, J. A. Hoefer, and R. W. Luecke, Michigan State University.

P49. Effect of feeding certain defined nutrients on cellulose digestion and volatile fatty acid concentration of the rumen. R. L. Salsbury, J. A. Hoefer, and R. W. Luecke, Michigan State University.

P50. The nutrition of ruminal cellulolytic bacteria.* M. P. Bryant and I. M. Robinson, Animal Husbandry Research Division, ARS, Beltsville, Maryland.

P51. Enzymatic activity of rumen fluid cellulases. R. W. Stanley and E. M Kesler, The Pennsylvania State University.

P52. Factors affecting energy metabolism in ruminants. R. E. Brown and C. L. Davis, University of Illinois.

P53. Utilization of carbohydrates posterior to the rumen-reticulum of the bovine. J. T. Huber and N. L. Jacobson, Iowa State University.

P54. Rumen volatile fatty acid production as affected by feeding frequency. K. L. Knox and G. M. Ward, Colorado State University.

P55. A parakeratosis condition of rumen epithelium produced by an all-concentrate ration. G. Vidacs and G. M. Ward, Colorado State University.

P56. The effect of dihydrotachysterol on the blood calcium and phosphorus levels of dairy cows. S. Badawi, T. H. Blosser, and R. E. Watts, Washington State University.

P57. The destruction of penicillin by rumen microorganisms. C. K. Smith, June Azukas, R. S. Emery, and C. F. Huffman, Michigan State University.

P58. Amino acid metabolism by rumen bacteria.* T. R. Lewis and R. S. Emery, Michigan State University.

## Tuesday, June 21

8:00–10:00 P.M.

Section A. **Genetics**

S. D. Musgrave, Chariman

Forestry Building, Auditorium

P59. Growth and productive performances of Holstein–Red Sindhi crossbreds and their Holstein-Friesian contemporaries. C. Branton and J. B. Frye, Jr., Louisiana State University, and R. E. McDowell, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P60. Genetic relationships among the first three lactations of Holstein cows. A. E. Freeman, Iowa State University.

P61. Analysis of deviations of dairy records from different contemporary averages. L. D. Van Vleck, T. Heidhues, and C. R. Henderson, Cornell University.

* To be read by title.

P62. Factors affecting the composition of individual samples of cow's milk. C. M. VON KROSIGK, G. A. RICHARDSON, AND J. O. YOUNG, Oregon State College.

P63. Genetic influences on the composition of cow's milk. C. M. VON KROSIGK, J. O. YOUNG, AND G. A. RICHARDSON, Oregon State College.

P64. Heritability estimates of longevity in Holstein-Friesian cattle. R. D. PLOWMAN AND R. F. GAALAAS, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P65. A comparison between expected and actual accuracy of sire proofs under the New York system. T. HEIDHUES, L. D. VAN VLECK, AND C. R. HENDERSON, Cornell University.

P66. Comparison of rating procedures used in evaluating sires. G. V. O'BLENESS, L. D. VAN VLECK, AND C. R. HENDERSON, Cornell University.

P67. Distributions and parameters of records of A.I. daughters. L. H. WADELL, L. D. VAN VLECK, AND C. R. HENDERSON, Cornell University.

8:00–10:00 A.M.

SECTION B. **Milk Secretion**

J. C. THOMPSON, Chairman

Forestry Building, Room 230

P68. Observations on the cholinesterase activity of bovine plasma and colostrum. J. E. MONTOURE AND T. L. FORESTER, Washington Agricultural Experiment Station, Pullman.

P69. A comparison of ratio factors for extending part-time milk and fat records. R. C. LAMB AND L. D. MCGILLIARD, Michigan State University.

P70. Repeatability estimates of butterfat test for partial lactations in a herd of Holstein cattle. A. C. LINNERUD AND J. D. DONKER, University of Minnesota.

P71. The effect of long-time feeding of chlortetracycline to dairy cattle on lactation responses. C. F. FOREMAN AND A. E. FREEMAN, Iowa State University.

P72. Effects of breed, sire, season, and type of forage on milk composition. J. M. RAKES, O. T. STALLCUP, AND J. S. HAYDEN, Arkansas Agricultural Experiment Station, Fayetteville.

P73. The influence of season of freshening on the milk yield and butterfat percentage of Holstein-Friesian and Jersey cows in Texas. M. A. BROWN, T. C. CARTWRIGHT, AND A. W. QURESHI, Texas A & M College.

P74. Repeatability estimates of monthly solids-not-fat tests. W. J. BRAKEL, Ohio State University.

P75. Udder capacity estimates as affected by rate of secretion and intramammary pressure. H. A. TUCKER, R. P. REECE, AND R. E. MATHER, New Jersey Agricultural Experiment Station, New Brunswick.

P76. The effect of prepartum infusions on milk production. H. STRUSS, W. E. PETERSEN, AND B. CAMPBELL, University of Minnesota.

9:00–10:00 A.M.

PROGRAM SPONSORED BY ASSOCIATION'S EDUCATION COMMITTEE
R. P. NIEDERMEIER, Chairman
Forestry Building, Room 305

10:15 A.M.–12:15 P.M.

GENERAL BUSINESS MEETING OF THE ASSOCIATION
A. C. FAY, Chairman
University Auditorium

2:00– 4:30 P.M.

SECTION A.
JOINT WITH EXTENSION SECTION

**Symposium: Milk Solids-Not-Fat**
G. W. TRIMBERGER, Chairman
Forestry Building, Auditorium

Testing methods and marketing aspects—protein vs. solids-not-fat. B. L. HERRINGTON, Cornell University.

Genetic and environmental factors affecting composition. J. E. LEGATES, North Carolina State College.

Regional projects and the extension viewpoint. S. N. GAUNT, University of Massachusetts.

Field testing for solids-not-fat and protein. R. E. ERB, U. S. ASHWORTH, AND L. J. MANUS, State College of Washington.

2:00– 4:30 P.M.

SECTION B. **Reproductive Physiology**
J. C. THOMPSON, Chairman
Forestry Building, Room 230

P77. Effect of feeding methyl testosterone on the reproductive system in bull calves. O. H. HORTON, Arkansas Agricultural Experiment Station, Fayetteville.

P78. Lactic dehydrogenase studies with cell-free extracts of bovine semen. H. P. BROQUIST AND J. R. LODGE, University of Illinois.

P79. Lactic acid production by sperm at 37° C. after storage at 5° C. M. H. EHLERS, Washington State University.

P80. $C^{14}$-fructose movement through the spermatozoan cell surface. R. J. FLIPSE AND J. L. FLEEGER, Pennsylvania Agricultural Experiment Station, University Park.

P81. The effect of various substrates and inhibitors on the respiration of bovine epididymal-like spermatozoa. C. N. GRAVES AND G. W. SALISBURY, University of Illinois.

P82. A simplified technique for maintaining isolated rabbit testes by perfusion. N. L. VANDEMARK AND L. L. EWING, University of Illinois.

P83. Effect of 5° C. storage in yolk-citrate on Feulgen-positive material (DNA) of sperm heads. G. W. SALISBURY, L. DE LA TORRE, W. J. BIRGE, AND J. R. LODGE, University of Illinois.

P84. Effect of storage temperatures of −79 and −196° C. on fertility of bull semen. B. W. PICKETT, A. K. FOWLER, D. G. GOSSLEE, AND W. A. COWAN, University of Connecticut.

P85. Sperm production of bulls electro-ejaculated at different frequencies. J. W. AUSTIN, E. W. HUPP, AND R. L. MURPHREE, University of Tennessee—Atomic Energy Commission Agricultural Research Laboratory, Oak Ridge.

P86. Sperm output and sperm reserves of unilaterally vasectomized Holstein bulls at various ejaculation frequencies. R. P. AMANN AND J. O. ALMQUIST, Pennsylvania State University.

P87. Quantitative testicular histology and theoretical sperm production of Holstein bulls. R. P. AMANN, The Pennsylvania State University.

P88. Fertilizing capacity of bovine sperm maintained at room temperatures in coconut milk extender. C. NORMAN, C. E. JOHNSON, I. D. PORTERFIELD, E. GOLDPERG, AND R. S. DUNBAR, JR., West Virginia University.

4:30– 5:30 P.M.

JOINT WITH EXTENSION SECTION

**Meeting of Superintendents of Official Testing**

RALPH BONEWITZ, Chairman
Forestry Building, Auditorium

## Wednesday, June 22

8:00 A.M.–12:00 M.

SECTION A. **General Physiology**

G. W. TRIMBERGER, Chairman
Forestry Building, Auditorium

P89. Comparative physiologic activity of thyroxine and triiodothyronine in cattle. B. N. PREMACHANDRA, G. W. PIPES, AND C. W. TURNER, University of Missouri.

P90. The extraction of estrogens and their metabolites from large samples of bovine feces. R. R. LEGAULT, MARJORIE L. HUNT, AND A. O. SHAW, Washington State University.

P91. Effect of hormone implants on growth and breeding efficiency of dairy heifers. DENNIS ARMSTRONG, GERALD WARD, AND E. J. CARROLL, Colorado State University.

P92. The uptake and excretion of cesium$^{134}$ and potassium$^{42}$ in lactating dairy cows. R. G. CRAGLE, University of Tennessee—Atomic Energy Commission, Agricultural Research Laboratory, Oak Ridge.

P93. Triglyceride structure of milk and other animal fats. R. D. MCCARTHY, STUART PATTON, AND LAURA EVANS, Pennsylvania State University.

P94. Variation in energy content of 4% FCM. G. E. HAWKINS, R. Y. CANNON, AND J. A. LITTLE, Auburn University.

P95. Further studies on the characterization of bovine mammary cells in tissue culture. K. E. EBNER, C. R. HOOVER, ELIZABETH HAGEMAN, AND B. L. LARSON, University of Illinois.

P96. Esophageal cannulation of the bovine. J. T. BLAKE, Utah State University.

P97. The level of acetate in bovine blood. JOHN R. SABINE AND B. CONNOR JOHNSON, University of Illinois.

P98. Correction for hemolysis in photometric methods for assaying sulfobromophthalein and phenolsulfonphthalein in the blood of cattle in tests for hepatic and renal function. R. R. ANDERSON AND J. P. MIXNER, New Jersey Agricultural Experiment Station, Sussex.

P99. Inactivation of oxytocin by bovine blood serum. P. W. ASCHBACHER, D. J. SEEMAN, AND C. JENSEN, North Dakota Agricultural College.

P100. Studies on the annual variation in blood ketone bodies. P. J. VAN SOEST, G. D. TURNER, H. W. HAWK, AND J. F. SYKES, Animal Husbandry Research Division, ARS, Beltsville, Maryland.

P101. The level of insulin-free activity in the blood serum of normal and ketotic cows. M. C. CALHOUN, C. R. RICHARDS, AND J. L. FLEEGER, University of Delaware.

P102. Some changes in the blood composition of dairy cows as associated with parturition. K. A. KENDALL AND K. E. HARSHBARGER, University of Illinois.

P103. The neutralization of certain bovine enteroviruses by colostrum, milk, and blood serum. D. O. CLIVER, T. M. LUDWICK, AND E. H. BOHL, Ohio State University.

P104. Rumen activity and other observations in young dairy calves. R. K. WAUGH, SAMUEL RAMIREZ, AND BERNARDINO RODRIGUEZ, The Rockefeller Foundation and Departamento de Investigaciones Agropecuarias, Miniagricultura, Colombia.

P105. The evaluation of udder characteristics of pre- and post-partum Holstein heifers. H. C. HINES, T. M. LUDWICK, E. W. BRUM, E. R. RADER, AND D. O. RICHARDSON, Ohio Agricultural Experiment Station, Wooster.

P106. The effect of kind and level of concentrates on udder edema. R. W. HEMKEN, EDSEL GAINER, AND R. F. DAVIS, University of Maryland.

P107. Physiological effects of tranquilizers in dairy animals. E. L. FARMER AND L. H. SCHULTZ, University of Wisconsin.

P108. Urea utilization studies in the bovine, using the re-entrant duodenal fistula technique.* P. W. MOE, C. F. HUFFMAN, G. H. CONNER, R. S. EMERY, AND C. A. LASSITER, Michigan State University.

P109. The effect of various metabolites on in vivo acetate metabolism.* C. L. DAVIS, R. E. BROWN, AND J. R. STAUBUS, University of Illinois.

8:00 A.M.–12:00 M.

SECTION B. **Nutrition**

S. D. MUSGRAVE, Chairman

Forestry Building, Room 230

P110. Methods of analysis of bovine excreta for gross energy. W. P. FLATT, H. F. LAWSON, D. R. GILLIAM, H. F. RIGHTER, D. R. WALDO, C. E. COP-

* To be read by title.

POCK, AND P. J. VAN SOEST, Animal Husbandry Research Division, ARS, Beltsville, Maryland.

P111. The effect of levulinic acid on the digestibility of cellulose and energy in oat straw and alfalfa hay fed to dairy heifers. D. E. WALDERN AND T. H. BLOSSER, Washington State University.

P112. Feeding molasses with hays to dairy heifers and milking cows. W. A. KING, C. C. BRANNON, AND H. J. WEBB, Clemson College, Clemson, South Carolina.

P113. Maintenance requirements of dairy cows. J. W. THOMAS AND L. A. MOORE, Animal Husbandry Research Division, ARS, Beltsville, Maryland.

P114. Effect of level and source of sulfur on the utilization of urea by dairy heifers. L. D. BROWN, R. S. EMERY, E. J. BENNE, AND C. A. LASSITER, Michigan State University.

P115. The effect of a modified sulfite waste liquor and of calcium gluconate on milk production. R. S. EMERY, C. K. SMITH, T. R. LEWIS, JUNE AZUKUS, AND L. D. BROWN, Michigan State University.

P116. The value of feeding aureomycin to lactating dairy cows under field conditions. L. D. BROWN AND C. A. LASSITER, Michigan State University.

P117. Disappearance of volatile acids from the omasum. R. P. JOHNSTON, E. M. KESLER, AND R. D. MCCARTHY, The Pennsylvania State University.

P118. The influence of sodium salts of volatile fatty acids on rumen development in the young calf. C. VAIR, G. M. WARD, R. D. FRANDSON, AND E. E. FLAMBOE, Colorado State University.

P119. Calf milk replacer studies: The effect of the physical state of added fat. A. S. HODGSON AND F. R. MURDOCK, Western Washington Experiment Station, Puyallup.

P120. Utilization of milk replacer rations containing urea by young dairy calves. C. A. LASSITER, G. D. RIEGLE, L. D. BROWN, AND E. J. BENNE, Michigan State University.

P121. Oleandomycin and oxytetracycline plus oleandomycin as growth stimulants for young dairy calves. N. D. MAGRUDER, W. B. HARDIE, W. C. SHERMAN, AND W. M. REYNOLDS, Chas. Pfizer & Co., Inc., Terre Haute, Indiana.

P122. Large-scale tests of feeding oxytetracycline to lactating dairy cows. N. D. MAGRUDER, C. E. LOFGREN, AND W. M. REYNOLDS, Chas. Pfizer & Co., Inc., Terre Haute, Indiana.

P123. Effect of spiramycin on the growth and well-being of young dairy calves. S. A. MONTICELLO AND L. L. RUSOFF, Louisiana Agricultural Experiment Station, Baton Rouge.

P124. Some physiological responses of dairy calves to certain carbohydrates when added to a milk replacer diet. SHRIRANG P. NETKE, K. E. GARDNER, AND K. A. KENDALL, University of Illinois.

* To be read by title.

P125. The value of hay and rumen inoculation in early weaning of calves. C. H. NOLLER AND I. A. DICKSON, Purdue University.

P126. B-vitamin requirements of calves fed a high carbohydrate diet. MAGNAR RONNING AND N. J. BENEVENGA, University of California.

P127. Glucose metabolism in the young dairy calf. C. L. DAVIS AND R. E. BROWN, University of Illinois.

P128. The digestibility of alfalfa hay as affected by partial replacement by carbohydrates.* E. A. KANE, Animal Husbandry Research Division, ARS, Beltsville, Maryland.

1:00– 4:00 P.M.

SECTIONS A AND B

JOINT WITH EXTENSION AND MANUFACTURING SECTIONS

Field House

**An Address: The Honorable Ezra Taft Benson, Secretary of Agriculture**

**Symposium: Facts, Fallacies, and Conflicts Affecting the Demand for Dairy Foods**

**Moderator:** W. D. KNOX, Hoard's Dairyman, Fort Atkinson, Wisconsin

Adulterants. M. R. STEPHENS, Food and Drug Administration, Washington, D.C.

Calcium and strontium[90]. MARGARET A. OHLSON, State University of Iowa.

Milk in relation to specific disease. ZOE E. ANDERSON, Wayne State University.

# EXTENSION SECTION

**Monday, June 20**

8:00–10:30 A.M.

**Dairy Records**

RALPH BONEWITZ, Chairman

Ag Science, Room 205

E1. Putting D.H.I.A. records to work. DAWSON JORDAN, Colorado State University.

**Panel Discussion**

New Developments in Sire Evaluation. Moderator: C. D. MCGREW, Ohio State University.

E2. New DHIA sire proofs. J. F. KENDRICK, Agricultural Research Service, USDA.

E3. Utilization of new sire proofs from standpoint of research worker. J. E. LEGATES, North Carolina State College.

* To be read by title.

E4. Utilization of new sire proofs from standpoint of sire analyst. CHARLES BALDWIN, Central Ohio Breeding Association, 1224 Alton-Darby Road, Columbus, Ohio.

E5. Utilization of new sire proofs from standpoint of stud manager. J. ELMO PACKER, Cache Valley Breeding Association, Logan, Utah.

E6. Reliability of environmental data collected under field conditions. E. L. CORLEY, E. E. HEIZER, AND C. E. KRAEMER, University of Wisconsin.

10:30 A.M.–12:00 M.

EXTENSION SECTION BUSINESS MEETING
C. W. NIBLER, Chairman
Agricultural Science, Room 205

Report of Dairy Records Committee
RALPH BONEWITZ, Chairman

Report of 4-H Club Committee
C. C. OLSON, Chairman

Reports of Teaching Methods Committee
LYMAN H. RICH, Chairman

Report of the Resolutions Committee
JOSEPH TAYLOR, Chairman

Report of Nominating Committee

1:00– 3:50 P.M.

**Teaching Methods**
LYMAN H. RICH, Chairman
Ag. Science, Room 205

E7. Salesmanship schools for DHIA supervisors. J. D. BURKE, Cornell University.

**Panel Discussion**

Preparing and Packaging Visual Aids for County Workers. R. E. BURLESON, Moderator, Federal Extension Service, USDA.

E8. Role of the dairy specialist in a changing agriculture. J. W. POU, University of Arizona.
Presentation and Discussion of Exhibits.

4:00– 6:00 P.M.

JOINT WITH PRODUCTION SECTION
(See Production Section)
Forestry Building Auditorium

**Tuesday, June 21**

8:00–10:00 A.M.

**4-H Program**
C. C. OLSON, Chairman
Ag. Science, Room 205

Report of the 4-H Club Committee
C. C. OLSON, North Dakota Agricultural College.

E9. The importance of type in dairy cattle breeding and management. NED BAYLEY, Agricultural Research Service, USDA.

E10. Appraising the value of showing and judging experiences in 4-H club work. ROBERT STEWART, American Guernsey Cattle Club.

10:15 A.M.–12:15 P.M.

GENERAL BUSINESS MEETING OF THE ASSOCIATION

A. C. FAY, Chairman

University Auditorium

2:00– 4:30 P.M.

JOINT WITH PRODUCTION SECTION

**Symposium: Milk Solids-Not-Fat**

(See Production Section)

Forestry Building Auditorium

4:30– 5:30 P.M.

JOINT WITH PRODUCTION SECTION

**Meeting of Superintendents of Official Testing**

Forestry Building Auditorium

E11. Accuracy determination of a new milk metering and sampling device. S. W. MEAD AND R. C. LABEN, University of California.

## Wednesday, June 22

8:00–10:00 A.M.

MARVIN SENGER, Chairman

Ag. Science, Room 205

E12. Probability of obtaining superior producing animals from varying quality of AB proved sires. H. WILMOT CARTER, Cornell University.

E13. The California Mastitis Testing (CMT) Program. KENNETH G. MCKAY, University of California.

10:00 A.M.–12:00 M.

**The Evaluation of Forages**

Ag. Science, Room 205

E14. Alfalfa hay quality as determined by fiber analysis. G. P. LOFGREEN, J. H. MEYER, AND V. L. MARBLE, University of California.

E15. Teaching quality through chemical analysis. T. C. BLALOCK, North Carolina State College.

E16. A forage evaluation program using the "artificial rumen." STANLEY N. GAUNT, University of Massachusetts.

1:00– 4:00 P.M.

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS

Field House

**An Address: The Honorable Ezra Taft Benson, Secretary of Agriculture.**

**Symposium: Facts, Fallacies, and Conflicts Affecting the Demand for Dairy Foods.**

**Moderator:** W. D. KNOX, Hoard's Dairyman, Fort Atkinson, Wisconsin.

Adulterants: M. R. STEPHENS, Food and Drug Administration, Washington, D.C.

Calcium and strontium$^{90}$. MARGARET A. OHLSON, State University of Iowa.

Milk in relation to specific diseases. ZOE E. ANDERSON, Wayne State University.

**Monday, June 20**

9:00 A.M. **Registration of Student Delegates and Advisors**

10:00 A.M. W. W. SNYDER, Presiding
Union Building, Room 226

**The Organization of the A.D.S.A. and the Place of the Student Branch Section.** A. C. FAY, President, A.D.S.A.

**The Purpose of the Student Branch Section.** W. W. SNYDER, Chairman, Student Affiliate Committee

**The Proposed Organization of the Student Branch Section.** PAUL M. REAVES, Chairman, Committee for Organization

**Appointment of Committees.**

11:30 A.M. **Luncheon with Certain A.D.S.A. Members**
Student Union

**Tuesday, June 21**

8:30 A.M. PAUL M. REAVES, Presiding
Union Building, Room 226

**Report of Nominating Committee.**

**Report of Constitution and By-Laws Committee.**

**Discussion of Type of Program for the 1961 Meeting.** Led by members of the Section Program Committee.

**Discussion of Activities of Local Student Branches.** Led by selected student delegates.

**Wednesday, June 22**

8:30 A.M. J. B. MICKLE, Presiding
Union Building, Room 226

**Discussion of Student Branch Exhibits.** By the Judges.

**Election of Student Branch Section Officers for 1960-61.**

**Committee Reports.**

**Meeting of Officers and Committee Members with Student Branch Committee.**

## STANDARDIZATION OF MEDIA

After many years of dedicated effort by a number of individuals and groups, the Microbiological Media Commission was incorporated in the State of Ohio on February 23, 1960, as a nonprofit corporation for the purposes of "the establishment of standardization and uniformity in the identity, composition, performance, and labeling of the more important microbiological culture media, ingredients, and reagents, in order to assure constant growth-promoting qualities for microorganisms so that results obtained in biological control and in investigations in different laboratories will be consistently reliable." This section resulted following resolutions or letters endorsing the formation of such a commission from organizations which included the American Dairy Science Association, Association of Official Agricultural Chemists, International Association of Milk and Food Sanitarians, and Society of American Bacteriologists.

The Commission was incorporated in the name of Luther A. Black, Harold F. Clark, and Joseph C. McCaffrey, and the following designated as the first trustees:

M. Thomas Bartram, chairman, Archie H. Robertson, vice-chairman, Harold F. Clark, secretary, Glen L. Hays, corresponding secretary, William J. Noble, treasurer, Luther A. Black, F. Wellington Gilcreas, Harry E. Goresline, Joseph C. McCaffrey, Wallace W. Sanderson, and Leander S. Stuart.

## REVISION OF BY-LAWS

It is proposed that Article II of the by-laws which involves the nomination and election of officers be amended as follows:

Section 2. The nominating committee shall consist of five members including the past president, one representative each from the extension, manufacturing, and production sections and one representing commercial interests. The JOURNAL OF DAIRY SCIENCE for October shall contain the names of the nominating committee and a statement from the chairman, inviting members to suggest names of candidates for office. These suggestions must be in the hands of the committee by January 1.

Section 3. The chairman of the nominating committee shall send the names of the nominees for each office with their photographs and biographical sketches to the editor by March 1 for publication in the JOURNAL OF DAIRY SCIENCE for April. Also, the chairman shall send duplicate copies of the biographies to the Secretary-Treasurer.

H. F. JUDKINS, Secretary-Treasurer
White Plains, New York.

# BOOK REVIEW

Les Bacteries Lactiques et les Laits Fermentes. E. Brochu, R. Riel, and C. Vezina. Monograph 3, Recherches Agronomiques, Le Conseil des Recherches Agricoles, Ministere de l'Agriculture de la Province de Quebec. 225 pp. 1959.

The first section of this monograph dealt with the influence of ten different lactic acid bacteria, a yogurt culture, and a kefir culture on the vitamin content of milk. The vitamins included thiamin, riboflavin, nicotinic acid, pantothenic acid, and ascorbic acid. The authors generally observed an initial drop in the vitamin content of the milk after the addition of the test microorganisms. Then, there was usually an increase in vitamin content with further incubation. The magnitude of the drop and of the recovery of the vitamins in milk varied greatly both with the vitamin studied and with the test organism.

A study of the bacteriostatic and bacteriocidal properties of fermented milks, including yogurt, buttermilk, acidophilus milk, and kefir, comprised the second section of this monograph. The authors concluded that the bacteriostatic properties of the fermented milks against 19 different assay organisms could be accounted for by the lactic acid formed.

The third section of this monograph was a study of the digestibility of fermented milks. Digestion was done in vitro, and simulated gastric digestion. The effects of homogenization, acidification, heating, and fermentation by microorganisms of yogurt, buttermilk, acidophilus milk, and kefir on digestibility were studied. All of these variables except homogenization increased digestibility.

In the appendix, an attempt was made to study thiamin and riboflavin synthesis by lactic acid bacteria in synthetic media. From this study, the authors concluded that more exact information concerning the nutrient requirements of lactic acid bacteria, and a more adequate synthetic medium, were needed before proper interpretation of the effect of lactic acid bacteria on the vitamin content of milk could be made.

In addition to the experimental results of the authors, and perhaps of more use, was the extensive literature survey for each topic in this monograph. Each experimental study was preceded by an excellent literature review of the subject investigated. These reviews were further augmented in the experimental portions by documented evaluations of the procedures and methods employed.

L. D. Witter
University of Illinois,
Urbana

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